```
  * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 12:37:40 ON 21 SEP 2004

=> fil .bec,canc
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       ESBIOBASE, BIOTECHNO, WPIDS, CANCERLIT' ENTERED AT 12:37:57 ON 21 SEP 2004
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

12 FILES IN THE FILE LIST

=> s hbx
FILE 'MEDLINE'
L1          375 HBX

FILE 'SCISEARCH'
L2          437 HBX

FILE 'LIFESCI'
L3          219 HBX

FILE 'BIOTECHDS'
L4           13 HBX

FILE 'BIOSIS'
L5          393 HBX

FILE 'EMBASE'
L6          324 HBX

FILE 'HCAPLUS'
L7          744 HBX

FILE 'NTIS'
L8           26 HBX

FILE 'ESBIOBASE'
L9          234 HBX

FILE 'BIOTECHNO'
L10         238 HBX

FILE 'WPIDS'
L11          16 HBX

FILE 'CANCERLIT'
L12         188 HBX

TOTAL FOR ALL FILES
L13        3207 HBX

=> s (hepatitis b virus or hbv)(8a)(inhibit? or treat?)
FILE 'MEDLINE'
        124564 HEPATITIS
        588054 B
        380675 VIRUS
         20080 HEPATITIS B VIRUS
                 (HEPATITIS(W)B(W)VIRUS)
         12596 HBV
       1126258 INHIBIT?
```

```
       2095022 TREAT?
L14       1891 (HEPATITIS B VIRUS OR HBV)(8A)(INHIBIT? OR TREAT?)

FILE 'SCISEARCH'
         90344 HEPATITIS
       1175884 B
        327078 VIRUS
         15490 HEPATITIS B VIRUS
                 (HEPATITIS(W)B(W)VIRUS)
         10506 HBV
        916162 INHIBIT?
       1542194 TREAT?
L15       1839 (HEPATITIS B VIRUS OR HBV)(8A)(INHIBIT? OR TREAT?)

FILE 'LIFESCI'
         22570 "HEPATITIS"
        196672 "B"
        188412 "VIRUS"
          8993 HEPATITIS B VIRUS
                 ("HEPATITIS"(W)"B"(W)"VIRUS")
          4790 HBV
        309773 INHIBIT?
        307130 TREAT?
L16        763 (HEPATITIS B VIRUS OR HBV)(8A)(INHIBIT? OR TREAT?)

FILE 'BIOTECHDS'
          4836 HEPATITIS
         52480 B
         45615 VIRUS
          2135 HEPATITIS B VIRUS
                 (HEPATITIS(W)B(W)VIRUS)
           603 HBV
         49081 INHIBIT?
         80652 TREAT?
L17        177 (HEPATITIS B VIRUS OR HBV)(8A)(INHIBIT? OR TREAT?)

FILE 'BIOSIS'
        105122 HEPATITIS
        686740 B
        498106 VIRUS
         26487 HEPATITIS B VIRUS
                 (HEPATITIS(W)B(W)VIRUS)
         12945 HBV
       1216079 INHIBIT?
       1744213 TREAT?
L18       1953 (HEPATITIS B VIRUS OR HBV)(8A)(INHIBIT? OR TREAT?)

FILE 'EMBASE'
         95843 "HEPATITIS"
        655511 "B"
        417150 "VIRUS"
         20763 HEPATITIS B VIRUS
                 ("HEPATITIS"(W)"B"(W)"VIRUS")
         10771 HBV
       1016504 INHIBIT?
       1953729 TREAT?
L19       1947 (HEPATITIS B VIRUS OR HBV)(8A)(INHIBIT? OR TREAT?)

FILE 'HCAPLUS'
         43952 HEPATITIS
       1452869 B
        305752 VIRUS
         11629 HEPATITIS B VIRUS
                 (HEPATITIS(W)B(W)VIRUS)
```

```
         7016 HBV
      1682892 INHIBIT?
      3084726 TREAT?
L20      1956 (HEPATITIS B VIRUS OR HBV)(8A)(INHIBIT? OR TREAT?)

FILE 'NTIS'
         1222 HEPATITIS
        66618 B
         7464 VIRUS
          124 HEPATITIS B VIRUS
                (HEPATITIS(W)B(W)VIRUS)
           84 HBV
        20337 INHIBIT?
       121081 TREAT?
L21         6 (HEPATITIS B VIRUS OR HBV)(8A)(INHIBIT? OR TREAT?)

FILE 'ESBIOBASE'
        21628 HEPATITIS
       304026 B
        95107 VIRUS
         4713 HEPATITIS B VIRUS
                (HEPATITIS(W)B(W)VIRUS)
         3879 HBV
       390402 INHIBIT?
       494402 TREAT?
L22       744 (HEPATITIS B VIRUS OR HBV)(8A)(INHIBIT? OR TREAT?)

FILE 'BIOTECHNO'
        27744 HEPATITIS
       228519 B
       178689 VIRUS
         8427 HEPATITIS B VIRUS
                (HEPATITIS(W)B(W)VIRUS)
         5147 HBV
       301415 INHIBIT?
       280839 TREAT?
L23       957 (HEPATITIS B VIRUS OR HBV)(8A)(INHIBIT? OR TREAT?)

FILE 'WPIDS'
        11712 HEPATITIS
      1147917 B
        36547 VIRUS
         1540 HEPATITIS B VIRUS
                (HEPATITIS(W)B(W)VIRUS)
          841 HBV
       221624 INHIBIT?
       908740 TREAT?
L24       458 (HEPATITIS B VIRUS OR HBV)(8A)(INHIBIT? OR TREAT?)

FILE 'CANCERLIT'
        21807 HEPATITIS
       140342 B
       121638 VIRUS
         5321 HEPATITIS B VIRUS
                (HEPATITIS(W)B(W)VIRUS)
         3543 HBV
       240873 INHIBIT?
       518305 TREAT?
L25       827 (HEPATITIS B VIRUS OR HBV)(8A)(INHIBIT? OR TREAT?)

TOTAL FOR ALL FILES
L26     13518 (HEPATITIS B VIRUS OR HBV)(8A)(INHIBIT? OR TREAT?)

=> s l13 and l26
```

```
FILE 'MEDLINE'
L27          36 L1 AND L14

FILE 'SCISEARCH'
L28          35 L2 AND L15

FILE 'LIFESCI'
L29          25 L3 AND L16

FILE 'BIOTECHDS'
L30           5 L4 AND L17

FILE 'BIOSIS'
L31          35 L5 AND L18

FILE 'EMBASE'
L32          32 L6 AND L19

FILE 'HCAPLUS'
L33          89 L7 AND L20

FILE 'NTIS'
L34           0 L8 AND L21

FILE 'ESBIOBASE'
L35          30 L9 AND L22

FILE 'BIOTECHNO'
L36          23 L10 AND L23

FILE 'WPIDS'
L37           6 L11 AND L24

FILE 'CANCERLIT'
L38          19 L12 AND L25

TOTAL FOR ALL FILES
L39         335 L13 AND L26

=> s l13(10a)inhibit?
FILE 'MEDLINE'
       1126258 INHIBIT?
L40          68 L1 (10A)INHIBIT?

FILE 'SCISEARCH'
        916162 INHIBIT?
L41          65 L2 (10A)INHIBIT?

FILE 'LIFESCI'
        309773 INHIBIT?
L42          55 L3 (10A)INHIBIT?

FILE 'BIOTECHDS'
         49081 INHIBIT?
L43           3 L4 (10A)INHIBIT?

FILE 'BIOSIS'
       1216079 INHIBIT?
L44          60 L5 (10A)INHIBIT?

FILE 'EMBASE'
       1016504 INHIBIT?
L45          67 L6 (10A)INHIBIT?
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```
FILE 'HCAPLUS'
       1682892 INHIBIT?
L46          88 L7 (10A)INHIBIT?

FILE 'NTIS'
         20337 INHIBIT?
L47           0 L8 (10A)INHIBIT?

FILE 'ESBIOBASE'
        390402 INHIBIT?
L48          61 L9 (10A)INHIBIT?

FILE 'BIOTECHNO'
        301415 INHIBIT?
L49          53 L10(10A)INHIBIT?

FILE 'WPIDS'
        221624 INHIBIT?
L50           3 L11(10A)INHIBIT?

FILE 'CANCERLIT'
        240873 INHIBIT?
L51          46 L12(10A)INHIBIT?

TOTAL FOR ALL FILES
L52         569 L13(10A) INHIBIT?

=> s src
FILE 'MEDLINE'
L53       14521 SRC

FILE 'SCISEARCH'
L54       13830 SRC

FILE 'LIFESCI'
L55        5487 SRC

FILE 'BIOTECHDS'
L56         256 SRC

FILE 'BIOSIS'
L57       14219 SRC

FILE 'EMBASE'
L58       10772 SRC

FILE 'HCAPLUS'
L59       14300 SRC

FILE 'NTIS'
L60        2012 SRC

FILE 'ESBIOBASE'
L61        7391 SRC

FILE 'BIOTECHNO'
L62        7046 SRC

FILE 'WPIDS'
L63         844 SRC

FILE 'CANCERLIT'
L64        8510 SRC
```

```
TOTAL FOR ALL FILES
L65       99188 SRC

=> s activat?
FILE 'MEDLINE'
L66      679531 ACTIVAT?

FILE 'SCISEARCH'
L67      746911 ACTIVAT?

FILE 'LIFESCI'
L68      215950 ACTIVAT?

FILE 'BIOTECHDS'
L69       24081 ACTIVAT?

FILE 'BIOSIS'
L70      697426 ACTIVAT?

FILE 'EMBASE'
L71      599361 ACTIVAT?

FILE 'HCAPLUS'
L72     1141473 ACTIVAT?

FILE 'NTIS'
L73       28010 ACTIVAT?

FILE 'ESBIOBASE'
L74      282083 ACTIVAT?

FILE 'BIOTECHNO'
L75      233622 ACTIVAT?

FILE 'WPIDS'
L76      243205 ACTIVAT?

FILE 'CANCERLIT'
L77      165622 ACTIVAT?

TOTAL FOR ALL FILES
L78     5057275 ACTIVAT?

=> s upstream
FILE 'MEDLINE'
L79       38787 UPSTREAM

FILE 'SCISEARCH'
L80       44802 UPSTREAM

FILE 'LIFESCI'
L81       26414 UPSTREAM

FILE 'BIOTECHDS'
L82        4473 UPSTREAM

FILE 'BIOSIS'
L83       44460 UPSTREAM

FILE 'EMBASE'
L84       34425 UPSTREAM

FILE 'HCAPLUS'
L85       59939 UPSTREAM
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FILE 'NTIS'
L86        6224 UPSTREAM

FILE 'ESBIOBASE'
L87       24184 UPSTREAM

FILE 'BIOTECHNO'
L88       28166 UPSTREAM

FILE 'WPIDS'
L89       61699 UPSTREAM

FILE 'CANCERLIT'
L90       10465 UPSTREAM

TOTAL FOR ALL FILES
L91      384038 UPSTREAM

=> s l65 and l78(5a)l91
FILE 'MEDLINE'
           2812 L66(5A)L79
L92         111 L53 AND L66(5A)L79

FILE 'SCISEARCH'
           2900 L67(5A)L80
L93         112 L54 AND L67(5A)L80

FILE 'LIFESCI'
           1752 L68(5A)L81
L94          43 L55 AND L68(5A)L81

FILE 'BIOTECHDS'
            176 L69(5A)L82
L95           0 L56 AND L69(5A)L82

FILE 'BIOSIS'
           2984 L70(5A)L83
L96         117 L57 AND L70(5A)L83

FILE 'EMBASE'
           2570 L71(5A)L84
L97          95 L58 AND L71(5A)L84

FILE 'HCAPLUS'
           3580 L72(5A)L85
L98         101 L59 AND L72(5A)L85

FILE 'NTIS'
             16 L73(5A)L86
L99           1 L60 AND L73(5A)L86

FILE 'ESBIOBASE'
           1976 L74(5A)L87
L100         83 L61 AND L74(5A)L87

FILE 'BIOTECHNO'
           1866 L75(5A)L88
L101         56 L62 AND L75(5A)L88

FILE 'WPIDS'
            249 L76(5A)L89
L102          0 L63 AND L76(5A)L89
```

```
FILE 'CANCERLIT'
          978 L77(5A)L90
L103       52 L64 AND L77(5A)L90

TOTAL FOR ALL FILES
L104      771 L65 AND L78(5A) L91

=> s l65 and (hbv or hbx)
FILE 'MEDLINE'
        12596 HBV
          375 HBX
L105       17 L53 AND (HBV OR HBX)

FILE 'SCISEARCH'
        10506 HBV
          437 HBX
L106       15 L54 AND (HBV OR HBX)

FILE 'LIFESCI'
         4790 HBV
          219 HBX
L107        9 L55 AND (HBV OR HBX)

FILE 'BIOTECHDS'
          603 HBV
           13 HBX
L108        1 L56 AND (HBV OR HBX)

FILE 'BIOSIS'
        12945 HBV
          393 HBX
L109       17 L57 AND (HBV OR HBX)

FILE 'EMBASE'
        10771 HBV
          324 HBX
L110       11 L58 AND (HBV OR HBX)

FILE 'HCAPLUS'
         7016 HBV
          744 HBX
L111       20 L59 AND (HBV OR HBX)

FILE 'NTIS'
           84 HBV
           26 HBX
L112        0 L60 AND (HBV OR HBX)

FILE 'ESBIOBASE'
         3879 HBV
          234 HBX
L113       10 L61 AND (HBV OR HBX)

FILE 'BIOTECHNO'
         5147 HBV
          238 HBX
L114        9 L62 AND (HBV OR HBX)

FILE 'WPIDS'
          841 HBV
           16 HBX
L115        5 L63 AND (HBV OR HBX)

FILE 'CANCERLIT'
```

```
          3543 HBV
           188 HBX
L116         8 L64 AND (HBV OR HBX)

TOTAL FOR ALL FILES
L117       122 L65 AND (HBV OR HBX)

=> s (l39 or l52 or l104 or l117) and py<=1998 range=2003,
FILE 'MEDLINE'
'2003,' IS NOT A VALID RANGE FOR FILE 'MEDLINE'
SEARCH ENDED BY USER

FILE 'SCISEARCH'
             0 PY<=1998
L118         0 (L28 OR L41 OR L93 OR L106) AND PY<=1998

FILE 'LIFESCI'
           304 PY<=1998
L119         0 (L29 OR L42 OR L94 OR L107) AND PY<=1998

FILE 'BIOTECHDS'
            61 PY<=1998
                 (PY<=1998)
L120         0 (L30 OR L43 OR L95 OR L108) AND PY<=1998

FILE 'BIOSIS'
          1185 PY<=1998
L121         0 (L31 OR L44 OR L96 OR L109) AND PY<=1998

FILE 'EMBASE'
           134 PY<=1998
L122         0 (L32 OR L45 OR L97 OR L110) AND PY<=1998

FILE 'HCAPLUS'
          3228 PY<=1998
L123         0 (L33 OR L46 OR L98 OR L111) AND PY<=1998

FILE 'NTIS'
          1700 PY<=1998
L124         0 (L34 OR L47 OR L99 OR L112) AND PY<=1998

FILE 'ESBIOBASE'
             0 PY<=1998
L125         0 (L35 OR L48 OR L100 OR L113) AND PY<=1998

FILE 'BIOTECHNO'
       1165535 PY<=1998
L126        46 (L36 OR L49 OR L101 OR L114) AND PY<=1998

FILE 'WPIDS'
          9788 PY<=1998
                 (PY<=1998)
L127         0 (L37 OR L50 OR L102 OR L115) AND PY<=1998

FILE 'CANCERLIT'
             0 PY<=1998
L128         0 (L38 OR L51 OR L103 OR L116) AND PY<=1998

TOTAL FOR ALL FILES
L129        46 (L39 OR L52 OR L104 OR L117) AND PY<=1998

=> fil medli
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                 ENTRY    SESSION
```

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FULL ESTIMATED COST                                    18.10      18.31

FILE 'MEDLINE' ENTERED AT 12:44:56 ON 21 SEP 2004

=> s (139 or 152 or 1104 or 1117) and py<=1998 range=2003000000,
         15308 PY<=1998
L130         0 (L27 OR L40 OR L92 OR L105) AND PY<=1998

=> fil .bec,canc
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.76      19.07

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       ESBIOBASE, BIOTECHNO, WPIDS, CANCERLIT' ENTERED AT 12:46:11 ON 21 SEP 2004
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

12 FILES IN THE FILE LIST

=> s cyclosporin a or csa
FILE 'MEDLINE'
         14326 CYCLOSPORIN
       7840549 A
         10282 CYCLOSPORIN A
                 (CYCLOSPORIN(W)A)
          9456 CSA
L131     16656 CYCLOSPORIN A OR CSA

FILE 'SCISEARCH'
         12731 CYCLOSPORIN
       9680070 A
          9389 CYCLOSPORIN A
                 (CYCLOSPORIN(W)A)
          8604 CSA
L132     15472 CYCLOSPORIN A OR CSA

FILE 'LIFESCI'
          5225 "CYCLOSPORIN"
       2040698 "A"
          4840 CYCLOSPORIN A
                 ("CYCLOSPORIN"(W)"A")
          2845 CSA
L133      5732 CYCLOSPORIN A OR CSA

FILE 'BIOTECHDS'
           291 CYCLOSPORIN
        325346 A
           193 CYCLOSPORIN A
                 (CYCLOSPORIN(W)A)
           133 CSA
L134        314 CYCLOSPORIN A OR CSA

FILE 'BIOSIS'
         19589 CYCLOSPORIN
       7737549 A
         14078 CYCLOSPORIN A
                 (CYCLOSPORIN(W)A)
          9668 CSA
L135     20352 CYCLOSPORIN A OR CSA

FILE 'EMBASE'
         61784 "CYCLOSPORIN"
       6805497 "A"
         35948 CYCLOSPORIN A
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```
                ("CYCLOSPORIN"(W)"A")
          9257 CSA
L136     39550 CYCLOSPORIN A OR CSA

FILE 'HCAPLUS'
         14277 CYCLOSPORIN
      18166895 A
         11791 CYCLOSPORIN A
                 (CYCLOSPORIN(W)A)
          7733 CSA
L137     15890 CYCLOSPORIN A OR CSA

FILE 'NTIS'
            33 CYCLOSPORIN
       1650689 A
            20 CYCLOSPORIN A
                 (CYCLOSPORIN(W)A)
           312 CSA
L138        330 CYCLOSPORIN A OR CSA

FILE 'ESBIOBASE'
          5092 CYCLOSPORIN
       2153196 A
          4012 CYCLOSPORIN A
                 (CYCLOSPORIN(W)A)
          3616 CSA
L139       6283 CYCLOSPORIN A OR CSA

FILE 'BIOTECHNO'
          9200 CYCLOSPORIN
       1454372 A
          6175 CYCLOSPORIN A
                 (CYCLOSPORIN(W)A)
          2041 CSA
L140       6770 CYCLOSPORIN A OR CSA

FILE 'WPIDS'
          1424 CYCLOSPORIN A
                 (CYCLOSPORIN)
           310 CSA
L141       1697 CYCLOSPORIN A OR CSA

FILE 'CANCERLIT'
          4063 CYCLOSPORIN
       1400898 A
          3272 CYCLOSPORIN A
                 (CYCLOSPORIN(W)A)
          2574 CSA
L142       4843 CYCLOSPORIN A OR CSA

TOTAL FOR ALL FILES
L143     133889 CYCLOSPORIN A OR CSA

=> s hepatitis b virus or hbv or hbx
FILE 'MEDLINE'
        124564 HEPATITIS
        588054 B
        380675 VIRUS
         20080 HEPATITIS B VIRUS
                 (HEPATITIS(W)B(W)VIRUS)
         12596 HBV
           375 HBX
L144     23236 HEPATITIS B VIRUS OR HBV OR HBX
```

```
FILE 'SCISEARCH'
         90344 HEPATITIS
       1175884 B
        327078 VIRUS
         15490 HEPATITIS B VIRUS
                 (HEPATITIS(W)B(W)VIRUS)
         10506 HBV
           437 HBX
L145     19369 HEPATITIS B VIRUS OR HBV OR HBX

FILE 'LIFESCI'
         22570 "HEPATITIS"
        196672 "B"
        188412 "VIRUS"
          8993 HEPATITIS B VIRUS
                 ("HEPATITIS"(W)"B"(W)"VIRUS")
          4790 HBV
           219 HBX
L146      9328 HEPATITIS B VIRUS OR HBV OR HBX

FILE 'BIOTECHDS'
          4836 HEPATITIS
         52480 B
         45615 VIRUS
          2135 HEPATITIS B VIRUS
                 (HEPATITIS(W)B(W)VIRUS)
           603 HBV
            13 HBX
L147      2166 HEPATITIS B VIRUS OR HBV OR HBX

FILE 'BIOSIS'
        105122 HEPATITIS
        686740 B
        498106 VIRUS
         26487 HEPATITIS B VIRUS
                 (HEPATITIS(W)B(W)VIRUS)
         12945 HBV
           393 HBX
L148     27887 HEPATITIS B VIRUS OR HBV OR HBX

FILE 'EMBASE'
         95843 "HEPATITIS"
        655511 "B"
        417150 "VIRUS"
         20763 HEPATITIS B VIRUS
                 ("HEPATITIS"(W)"B"(W)"VIRUS")
         10771 HBV
           324 HBX
L149     22727 HEPATITIS B VIRUS OR HBV OR HBX

FILE 'HCAPLUS'
         43952 HEPATITIS
       1452869 B
        305752 VIRUS
         11629 HEPATITIS B VIRUS
                 (HEPATITIS(W)B(W)VIRUS)
          7016 HBV
           744 HBX
L150     12574 HEPATITIS B VIRUS OR HBV OR HBX

FILE 'NTIS'
          1222 HEPATITIS
         66618 B
          7464 VIRUS
```

```
           124 HEPATITIS B VIRUS
                 (HEPATITIS(W)B(W)VIRUS)
            84 HBV
            26 HBX
L151       184 HEPATITIS B VIRUS OR HBV OR HBX

FILE 'ESBIOBASE'
         21628 HEPATITIS
        304026 B
         95107 VIRUS
          4713 HEPATITIS B VIRUS
                 (HEPATITIS(W)B(W)VIRUS)
          3879 HBV
           234 HBX
L152       5630 HEPATITIS B VIRUS OR HBV OR HBX

FILE 'BIOTECHNO'
         27744 HEPATITIS
        228519 B
        178689 VIRUS
          8427 HEPATITIS B VIRUS
                 (HEPATITIS(W)B(W)VIRUS)
          5147 HBV
           238 HBX
L153       9076 HEPATITIS B VIRUS OR HBV OR HBX

FILE 'WPIDS'
         11712 HEPATITIS
       1147917 B
         36547 VIRUS
          1540 HEPATITIS B VIRUS
                 (HEPATITIS(W)B(W)VIRUS)
           841 HBV
            16 HBX
L154       1817 HEPATITIS B VIRUS OR HBV OR HBX

FILE 'CANCERLIT'
         21807 HEPATITIS
        140342 B
        121638 VIRUS
          5321 HEPATITIS B VIRUS
                 (HEPATITIS(W)B(W)VIRUS)
          3543 HBV
           188 HBX
L155       6006 HEPATITIS B VIRUS OR HBV OR HBX

TOTAL FOR ALL FILES
L156     140000 HEPATITIS B VIRUS OR HBV OR HBX

=> s l143 and l156
FILE 'MEDLINE'
L157         42 L131 AND L144

FILE 'SCISEARCH'
L158         32 L132 AND L145

FILE 'LIFESCI'
L159         10 L133 AND L146

FILE 'BIOTECHDS'
L160          1 L134 AND L147

FILE 'BIOSIS'
L161         58 L135 AND L148
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FILE 'EMBASE'
L162       172 L136 AND L149

FILE 'HCAPLUS'
L163        16 L137 AND L150

FILE 'NTIS'
L164         0 L138 AND L151

FILE 'ESBIOBASE'
L165        11 L139 AND L152

FILE 'BIOTECHNO'
L166        39 L140 AND L153

FILE 'WPIDS'
L167         6 L141 AND L154

FILE 'CANCERLIT'
L168        15 L142 AND L155

TOTAL FOR ALL FILES
L169       402 L143 AND L156

=> s bapta or cgp37157
FILE 'MEDLINE'
          2518 BAPTA
            13 CGP37157
L170      2531 BAPTA OR CGP37157

FILE 'SCISEARCH'
          2448 BAPTA
            13 CGP37157
L171      2461 BAPTA OR CGP37157

FILE 'LIFESCI'
           890 BAPTA
             7 CGP37157
L172       897 BAPTA OR CGP37157

FILE 'BIOTECHDS'
             4 BAPTA
             0 CGP37157
L173         4 BAPTA OR CGP37157

FILE 'BIOSIS'
          3133 BAPTA
            17 CGP37157
L174      3148 BAPTA OR CGP37157

FILE 'EMBASE'
          2388 BAPTA
            13 CGP37157
L175      2401 BAPTA OR CGP37157

FILE 'HCAPLUS'
          2630 BAPTA
            17 CGP37157
L176      2647 BAPTA OR CGP37157

FILE 'NTIS'
            14 BAPTA
             0 CGP37157
```

```
L177        14 BAPTA OR CGP37157

FILE 'ESBIOBASE'
          1727 BAPTA
            13 CGP37157
L178      1739 BAPTA OR CGP37157

FILE 'BIOTECHNO'
           604 BAPTA
             3 CGP37157
L179       607 BAPTA OR CGP37157

FILE 'WPIDS'
            39 BAPTA
             0 CGP37157
L180        39 BAPTA OR CGP37157

FILE 'CANCERLIT'
           426 BAPTA
             0 CGP37157
L181       426 BAPTA OR CGP37157

TOTAL FOR ALL FILES
L182     16914 BAPTA OR CGP37157

=> s l182 and l156
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FILE 'SCISEARCH'
L184         1 L171 AND L145

FILE 'LIFESCI'
L185         0 L172 AND L146

FILE 'BIOTECHDS'
L186         0 L173 AND L147

FILE 'BIOSIS'
L187         1 L174 AND L148

FILE 'EMBASE'
L188         1 L175 AND L149

FILE 'HCAPLUS'
L189         1 L176 AND L150

FILE 'NTIS'
L190         0 L177 AND L151

FILE 'ESBIOBASE'
L191         0 L178 AND L152

FILE 'BIOTECHNO'
L192         1 L179 AND L153

FILE 'WPIDS'
L193         0 L180 AND L154

FILE 'CANCERLIT'
L194         0 L181 AND L155

TOTAL FOR ALL FILES
L195         6 L182 AND L156
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L196         25 (L157 OR L183) NOT 1999-2004/PY

FILE 'SCISEARCH'
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L197         20 (L158 OR L184) NOT 1999-2004/PY

FILE 'LIFESCI'
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L198          4 (L159 OR L185) NOT 1999-2004/PY

FILE 'BIOTECHDS'
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L199          0 (L160 OR L186) NOT 1999-2004/PY

FILE 'BIOSIS'
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L200         36 (L161 OR L187) NOT 1999-2004/PY

FILE 'EMBASE'
       2611185 1999-2004/PY
L201         72 (L162 OR L188) NOT 1999-2004/PY

FILE 'HCAPLUS'
       5451771 1999-2004/PY
L202          5 (L163 OR L189) NOT 1999-2004/PY

FILE 'NTIS'
         99012 1999-2004/PY
L203          0 (L164 OR L190) NOT 1999-2004/PY

FILE 'ESBIOBASE'
       1645081 1999-2004/PY
L204          5 (L165 OR L191) NOT 1999-2004/PY

FILE 'BIOTECHNO'
        611346 1999-2004/PY
L205         20 (L166 OR L192) NOT 1999-2004/PY

FILE 'WPIDS'
       4833163 1999-2004/PY
L206          1 (L167 OR L193) NOT 1999-2004/PY

FILE 'CANCERLIT'
        354668 1999-2004/PY
L207         11 (L168 OR L194) NOT 1999-2004/PY

TOTAL FOR ALL FILES
L208        199 (L169 OR L195) NOT 1999-2004/PY

=> dup rem l208
PROCESSING COMPLETED FOR L208
L209         101 DUP REM L208 (98 DUPLICATES REMOVED)

=> d ab 6, 16, 24, 52, 62, 66, 71, 80, 84

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AB We report the case of a 51-year-old renal transplant patient, treated by interferon alpha (5MUI, three times a week) since he presented a coinfection by hepatitis B (**HBV**) and hepatitis C (HCV) virus for more than 7 years, associated with a chronic increase in serum alanine aminotransferase (ALT) levels and a chronic active hepatitis. The 4-month treatment was associated with a sustained normalization of ALT, a disappearance of **HBV** replication and a transient clearance of HCV viremia. Side effects were moderate and included thrombopenia (90,000/mm3), leucopenia (2200/mm3), an increase in serum creatinine (178 μmol/l). The withdrawal of alpha interferon was associated with the correction of these parameters. No rejection was observed on kidney biopsy. Meanwhile, liver histology was not affected by the treatment. To date, nineteen months after the end of alpha interferon therapy **HBV** DNA was still negative; ALT remained normal despite the early recurrence of HCV viremia; this emphasized the fact that **HBV** infection was certainly the most important factor involved in the patient's chronic hepatitis. it is concluded that alpha interferon therapy is able to decline **HBV** replication for a prolonged period in renal transplant patient although its use should be performed with caution due to the potential renal side effects.

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AB We have studied the roles of immunoprophylaxis, patient selection policy and coexistent hepatitis D virus infection in the outcome of 56 HBsAg-positive elective liver transplant recipients. Twenty-nine unselected patients not treated with immunoprophylaxis formed group 1 and were compared to a recent consecutive series of 27 patients (group 2) in whom pre-transplant serological status was determined and who received immunoprophylaxis. One-year actuarial HBsAg serological recurrence rates were 48% in group 2 and 90% in group 1 with particular improvement in recipients who were either **HBV** DNA-negative or who had co-existent hepatitis delta virus infection. One-year patient survival has improved from 62% in group 1 to 86% in group 2 with improvements in hepatitis delta virus-negative and replicating recipients. Patients who have either co-existent hepatitis delta virus infection or are in group 2 have 1-year survival rates comparable to elective HBsAg-negative recipients (19/21 (90%), 22/27 (86%) vs 87%, respectively). In the event of recurrence, severe graft injury is diminished in recent patients and in those with coexistent delta infection who also have lower levels of circulating **HBV** DNA. Retransplantation for associated graft injury has a poor prognosis irrespective of administration of immunoprophylaxis. In elective liver recipients, immunoprophylaxis and/or hepatitis delta virus infection modulate **hepatitis B virus** recurrence and associated graft injury with consequent improvement in patient survival.

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L209 ANSWER 66 OF 101 MEDLINE on STN DUPLICATE 27

AB Four woodchucks chronically infected with hepatitis delta virus (HDV) were treated with **cyclosporin-A** (CyA) for 11 weeks. All animals had detectable HDAg in the liver and two of them were also positive for HDAg and HDV-RNA in serum. Reappearance of HDV in serum was noted in one of the two non-viraemic animals and increased viraemia in the two viraemic. HDV-RNA levels became elevated within a week of starting treatment and an inverse relationship between HDV-RNA and WHV-DNA became apparent during the treatment period. With discontinuation of treatment, HDV-RNA levels either returned to pretreatment levels or became negative. The remaining animal showed no return of viraemia during CyA treatment;

HDV-RNA remained negative and WHV-DNA levels did not change. Liver biopsies revealed a slight increase in lobular activity during CyA treatment in the animals showing increased viraemia. These data are consistent with the hypothesis that the host immune response exerts a negative control on the level of HDV viraemia and that HDV influences **HBV** replication independently of the host immune response. In an animal that may have been clearing HDV, immunosuppression did not result in recurrence of viraemia.

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AB Seven patients who underwent liver transplantation for HBsAg-positive, HDV-related liver disease were studied before and after surgery in a follow-up period ranging from 12 to 34 months. Markers of **HBV** and HDV infection were detected in serum and liver samples at least once a month. Serum HDV RNA was detected by using a slot hybridization analysis of serum samples with a single-stranded RNA probe. Before liver grafting, 4/7 patients were serum HDV RNA positive. After surgery, 7/7 patients had recurrent delta infection. Serum HDAg, liver HDAg and anti-HD IgM were identified in 3/7, 6/7 and 5/7 patients respectively. Serum HDV RNA was detected in all 7 patients. In subjects with recurrent hepatitis (3/7) serum HDV RNA was persistently detected, whereas, in the four patients who did not develop any sign of hepatitis, three had a transient delta viraemic phase and one was persistently HDV RNA positive. With regard to **HBV** markers, before liver grafting all patients were **HBV** DNA negative. After surgery, HBsAg and **HBV** DNA were persistently shown in 4/7 patients with continuous HDV replication. In the remaining three, HDV RNA was detectable despite the absence of HBsAg. This study indicates that virological analysis of reinfection of the new liver in transplanted, immunocompromised patients may possible offer important information not only for a better choice of treatment of these patients, but also for a better appraisal of the natural history of HDV infection, such as the interactions between HDV and **HBV** replication or the pathogenic mechanisms of liver damage.

=> log y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	90.39	109.46

STN INTERNATIONAL LOGOFF AT 13:11:03 ON 21 SEP 2004

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L3	26	1 and 2	US-PGPUB; USPAT	OR	OFF	2004/09/21 09:07
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L14	798	5 near5 6	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:27
L15	55	12 and 14	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:27
L16	11548	cyclosporin or csa	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:48
L17	2	16 same 12	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:49
L18	417	bapta or cgp37157	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:52
L19	6	18 and 12	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:52

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PGPUB-DOCUMENT-NUMBER: 20040138119

PGPUB-FILING-TYPE: new

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TITLE: Use of hepatitis B X-interacting protein (HBXIP) in modulation of apoptosis

PUBLICATION-DATE: July 15, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 665975

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non-provisional-of-provisional 60412109 20020918 US

US-CL-CURRENT: 514/12

ABSTRACT:

Novel methods of regulating cellular apoptosis by affecting the interaction of hepatitis B X-interacting protein (HBXIP) with Survivin are described. More specifically, these novel methods of enhancing apoptosis of neoplastic cells comprises inhibiting interaction of hepatitis B X-interacting protein (HBXIP) with Survivin.

RELATED APPLICATIONS

[0001] This application claims the benefit of priority under 35 U.S.C. 119(e) of the U.S. Provisional Application 60/412,109 filed Sep. 18, 2002, the disclosure of which is expressly incorporated herein by reference in its entirety.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (7):
[0007] HBXIP was originally isolated as a human protein which binds the viral oncogenic protein, HBX, of the Hepatitis B Virus (HBV) (Melegari, M. et al. 1998 J Virol 72:1737-1743). HBXIP encodes a protein of 9.6-kDa with a putative leucine zipper motif. Expression of HBXIP mRNA has been demonstrated in essentially all tissues examined to date, and is not limited to the liver (Melegari, M. et al. 1998 J Virol 72:1737-1743). In the context of HBV-infection, HBXIP reportedly reduces viral replication and abolishes the transactivation function of viral HBX protein (Melegari, M. et al. 1998 J Virol 72:1737-1743), however, little is known about the physiological roles of HBXIP in human cells.

Summary of Invention Paragraph - BSTX (11):
[0010] Another aspect of the invention includes a pharmaceutical composition

comprising a compound that inhibits HBXIP in the presence of Survivin. Yet another embodiment is a method of treating neoplastic disease comprising administration of an inhibitor of HBXIP in the presence of Survivin. A method for treating human liver disease associated with HBV is also described, comprising administration of an inhibitor of HBXIP in the presence of Survivin.

Brief Description of Drawings Paragraph - DRTX (7):

[0016] FIG. 6(a-f) shows that HBX associates with Survivin through HBXIP and suppresses Caspase activation. FIG. 6a shows immunoblot analysis using anti-HBX antibody of in vitro protein binding after incubation of recombinant His.sub.6-HBX with GST-HBXIP, GST-Survivin, or GST-CD40 (control). FIGS. 6b and 6c show caspase activity measured in 293 cell extracts in the presence of His.sub.6-HBX or control [CTR] proteins, purified His.sub.6-HBXIP, purified Survivin, or various combinations of these proteins after Cytochrome c and dATP were added. FIG. 6d shows the results of immunoprecipitation using anti-Survivin antibody (upper panel) of lysates of HEK 293 cell expressing FLAG-tagged-HBX or FLAG-SIP (as a control) together with Myc-Survivin or HA-HBXIP with anti-FLAG epitope antibody demonstrates increased association of Survivin with HBX when HBXIP was co-expressed (compare last two lanes at right). FIG. 6e shows the results when His.sub.6-pro-Caspase-9 was incubated with GST-HBXIP(+) or GST-CD40 control protein (-), in the presence or absence of His.sub.6-HBX and untagged Survivin. GST-fusion proteins were recovered on glutathione-Sepharose and bound proteins were detected by immunoblotting using anti-Caspase-9, anti-Survivin, or anti-HBX antisera. FIG. 6f shows immunoblotting using anti-Survivin antibody (top panel) of HepG2 cell extracts immunodepleted using anti-Survivin antisera or preimmune serum (CTR). Then extracts were analyzed for Caspase activity based on Ac-DEVD-AFC cleavage, where lysates were incubated with recombinant HBX (+) or control protein (-) prior to stimulation with Cytochrome c and dATP.

Detail Description Paragraph - DETX (2):

[0018] Survivin is an anti-apoptotic protein of undefined mechanism, which is pathologically over-expressed in most human cancers. It has been discovered that Survivin forms complexes with an endogenous cellular protein, Hepatitis B X-interacting protein (HBXIP), which was originally recognized for its association with the X protein of Hepatitis B Virus (HBX). Survivin/HBXIP complexes, but neither Survivin nor HBXIP individually, bind pro-Caspase-9, preventing its recruitment to Apaf1, and thereby selectively suppress apoptosis initiated via the mitochondria/Cytochrome C pathway. Viral HBX protein also interacts with the Survivin/HBXIP complex and suppresses Caspase activation in a Survivin-dependent manner. Thus, HBXIP functions as a cofactor for Survivin, and serves as a link between the cellular apoptosis machinery and a viral pathogen involved on an epidemic scale in hepatocellular carcinogenesis.

Detail Description Paragraph - DETX (3):

[0019] In an effort to provide insights into the anti-apoptotic mechanism of Survivin, cDNA libraries were screened for Survivin-binding proteins, resulting in the discovery that hepatitis B X-interacting protein (HBXIP) associates with p17 Survivin. HBXIP was originally identified by virtue of its ability to interact with the HBX protein of Hepatitis B Virus (HBV) (Melegari, M. et al. 1998 J Virol 72:1737-1743). HBX is a putative oncogenic protein, which has been previously implicated in regulation of apoptosis, as well as other processes (reviewed in Murakami, S. 2001 J Gastroenterol 36:651-660). HBXIP operates as a cofactor for Survivin, allowing it to bind and suppress activation of pro-Caspase-9, the apical protease in a mitochondrial pathway for cell death. These findings thus provide novel insights into the anti-apoptotic mechanism of Survivin, and provide a link between Survivin and neoplastic diseases associated with HBV infection.

Detail Description Paragraph - DETX (142):
Cloning of Survivin, HBXIP and HBX and Production of Recombinant Proteins

Detail Description Paragraph - DETX (143):
[0153] cDNA cloning and plasmid construction. A cDNA encoding human HBXIP was generated by reverse transcription from Jurkat T cell mRNA using SuperScript II (Gibco, Rockville, Md.), followed by the amplification using the Expand High Fidelity PCR system (Roche, Mannheim, Germany) and oligonucleotide primers as follows: 5'-GACGAATTCATGGAGGCGACCTTGGAGCA-3' (forward) (SEQ ID NO: 5) and 5'-GATCTCGAGTCAAGAGGCCATTTTGTGCA-3' (reverse) (SEQ ID NO: 6). The resultant cDNA fragments were ligated into the plasmid pcDNA3-FLAG for mammalian expression (Matsuzawa, S. & Reed, J. C. 2001 Mol Cell 7:915-926), or into pET21d-N-His.sub.6 and pGEX4T-1 for expression in bacteria. Various fragments of Survivin cDNA were also PCR-amplified from pcDNA3-Survivin (Tamm, I. et al. 1998 Cancer Res 58:5315-5320), and subcloned into pcDNA3 (Invitrogen, Carlsbad, Calif.). The gene encoding HBX was synthesized by PCR from DNA obtained from patients with hepatocellular carcinoma, as described previously (Marusawa, H. et al. 2000 Hepatology 31:488-495).

Detail Description Paragraph - DETX (144):
[0154] Production of recombinant proteins and in vitro protein binding assays. Recombinant proteins were purified essentially as described (Deveraux, Q. L. & Reed, J. C. 1999 Genes Dev 13:239-252; Zou, H. et al. 1999 J Biol Chem 274:11549-11556). His.sub.6-HBX protein was made using the Rapid Translation System (Roche), and 2 .mu.l of synthesized reaction mixture was used for Caspase assays. Purified GST-fusion protein or His.sub.6-tagged proteins immobilized on glutathione-Sepharose beads or nickel beads, respectively, were incubated in 1% Triton-X 100/PBS for 1 h at 4.degree. C. Then, the beads were washed with binding buffer (5 mM MgCl.sub.2, 10% glycerol, 0.5 mg/ml BSA, 5 mM 2-mercaptoethanol and 50 mM Tris-Cl, pH 7.5) and incubated overnight at 4.degree. C. with various recombinant proteins or in vitro translated-.sup.35S-labeled proteins produced using TNT-coupled reticulocyte lysates (Promega, Madison, Wis.). Protein on beads were washed four times in binding buffer, and bound proteins were eluted in SDS sample buffer, and subjected to SDS-PAGE, as described previously (Deveraux, Q. L. & Reed, J. C. 1999 Genes Dev 13:239-252).

Detail Description Paragraph - DETX (159):
[0163] Polyclonal antisera specific for HBXIP were generated in rabbits using purified recombinant His.sub.6-HBXIP as an immunogen. Rabbit polyclonal antibody against pro-Caspase-9, pro-Caspase-3, XIAP and Survivin have been described (Krajewski, S. et al. 1999 PNAS USA 96:5752-5757; Tamm, I. et al. 1998 Cancer Res 58:5315-5320; Krajewska, M. et al. 1997 Cancer Res 57:1605-1613). Rabbit polyclonal antibody recognizing human Apaf1 was purchased from Cayman Chemical Company (Ann Arbor, Mich.). Mouse monoclonal antibodies against active Caspase-9 and Cytochrome C were purchased from Pharmingen (San Diego, Calif.). Rabbit polyclonal anti-HBX antibody was generously provided by Robert J. Schneider (New York University Medical School).

Detail Description Paragraph - DETX (192):
HBX Protein Collaborates With HBXIP in Suppressing Caspase Activation

Detail Description Paragraph - DETX (193):
[0183] The viral oncoprotein HBX is encoded in the HBV genome, and has been implicated in hepatocellular carcinogenesis through uncertain mechanisms (reviewed in Murakami, S. 2001 J Gastroenterol 36:651-660). In vitro protein binding assays were performed in which recombinant His.sub.6-HBX was incubated

with GST-HBXIP, GST-Survivin, or GST-CD40 (control) immobilized on glutathione-Sepharose. Bound proteins were analyzed by immunoblotting using anti-HBX antibody. This confirmed previously reported observations that HBX associates with HBXIP (FIG. 6a). HBX, in contrast, did not bind Survivin.

Detail Description Paragraph - DETX (194):

[0184] The ability of HBX to associate with HBXIP raised the question of whether this viral oncogenic protein could effect Caspases. Since attempts to produce soluble recombinant HBX in bacteria failed, in vitro translation using bacterial extracts was used for production of this viral protein. His.sub.6-HBX or control [CTR] proteins, purified His.sub.6-HBXIP, purified Survivin, or various combinations of these proteins were added to 293 cell extracts normalizing all samples for total protein added using control recombinant proteins, then Cytochrome c and dATP were added, and Caspase activity was measured 30 min later based on hydrolysis of Ac-DEVD-AFC. Release from fluorogenic AFC from Ac-DEVD-AFC was measured continuously. Addition of HBX protein to lysates prepared from HepG2 hepatocellular cancer cells suppressed Caspases activation induced by Cytochrome C and dATP, while control proteins prepared in the same manner had little effect (FIG. 6b). In addition, this viral protein augmented the inhibitory effect of recombinant purified HBXIP and Survivin on Cytochrome C-mediated Caspase activation (FIG. 6c), prompting examination into whether HBX protein associates with HBXIP/Survivin complexes.

Detail Description Paragraph - DETX (195):

[0185] For this purpose, co-immunoprecipitation assays were carried out using epitope-tagged HBX, HBXIP and Survivin expressed by transient transfection in HEK293T cells. Since HBX does not directly bind Survivin (FIG. 6a), it was reasoned that over-expressing HBXIP would increase the amounts of Survivin immunoprecipitated with HBX by bridging these two proteins together. HEK293 cells were transiently transfected with plasmids encoding FLAG-tagged-HBX or FLAG-SIP (as a control) together with Myc-Survivin or HA-HBXIP or control plasmid. Lysates were subjected to immunoprecipitation using anti-FLAG epitope antibody, demonstrating increased association of Survivin with HBX when HBXIP was co-expressed (compare last two lanes at right). Immunoprecipitates were analyzed by immunoblotting using anti-Survivin antibody (upper panel). Lysates were also analyzed by immunoblotting using anti-HBX (middle panel) or anti-HA antibodies (lower panel), confirming production of the intended proteins. Indeed, when HBX and Survivin were co-expressed with HBXIP, considerably more Survivin was associated with HBX-immunoprecipitates (FIG. 6d). These data therefore are consistent with the idea that HBX, HBXIP, and Survivin form complexes.

Detail Description Paragraph - DETX (196):

[0186] To address whether Survivin/HBXIP complexes were still capable of binding pro-Caspase-9 in the presence of HBX, we performed in vitro protein interaction assays, examining the association of pro-Caspase-9 with GST-HBXIP when Survivin, HBX, or both proteins were added. His.sub.6-pro-Caspase-9 was incubated with GST-HBXIP(+) or GST-CD40 control protein (-), in the presence or absence of His.sub.6-HBX and untagged Survivin. GST-fusion proteins were recovered on glutathione-Sepharose and bound proteins were detected by immunoblotting using anti-Caspase-9, anti-Survivin, or anti-HBX antisera. As shown above, GST-HBXIP pulled-down pro-Caspase-9 when Survivin was included in the binding assays (FIG. 6e lane 3). Addition of HBX did not impair pro-Caspase-9 association with GST-HBXIP, and instead increased pro-Caspase-9 binding slightly (FIG. 6e lane 4). Thus, rather than competing for binding to HBXIP, the HBX protein appears to form complexes with HBXIP in a manner that does not preclude simultaneous association with Survivin and pro-Caspase-9, and which may even enhance formation of multiprotein complexes containing these

proteins.

Detail Description Paragraph - DETX (197):
[0187] To further explore whether HBX suppresses Caspase activity through a Survivin-dependent mechanism, endogenous Survivin was immunodepleted from HepG2 cell lysates using anti-Survivin antisera or preimmune serum (CTR) and then equivalent amounts analyzed by immunoblotting using anti-Survivin antibody (top panel). Further, equivalent volumes of extracts were analyzed for Caspase activity based on Ac-DEVD-AFC cleavage, where lysates were incubated with recombinant HBX (+) or control protein (-) prior to stimulation with Cytochrome c and dATP. In extracts subjected to mock immunodepletion, HBX suppressed Caspase activation by roughly half (FIG. 6f). In contrast, when Survivin was immunodepleted, it was found that Cytochrome C was more potent at activating Caspases and that HBX had little inhibitory activity (FIG. 6f). Thus, HBX fails to inhibit Caspases in the absence of Survivin.

Detail Description Paragraph - DETX (201):
[0189] HBXIP was originally isolated as a human protein which binds the viral oncogenic protein, HBX, of the Hepatitis B Virus (HBV) (Melegari, M. et al. 1998 J Virol 72:1737-1743). HBXIP encodes a protein of 9.6-kDa with a putative leucine zipper motif. Expression of HBXIP mRNA has been demonstrated in essentially all tissues examined to date, and is not limited to the liver (Melegari, M. et al. 1998 J Virol 72:1737-1743). In the context of HBV-infection, HBXIP reportedly reduces viral replication and abolishes the transactivation function of viral HBX protein (Melegari, M. et al. 1998 J Virol 72:1737-1743), however, little is known about the physiological roles of HBXIP in human cells. The cellular HBXIP protein is conserved in mice and rodents, suggesting an evolutionarily conserved function. Based on the data presented here, HBXIP is envisioned to be an anti-apoptotic co-factor of Survivin. Consistent with this idea, siRNA-mediated reductions in endogenous HBXIP levels sensitized cells to apoptosis, while over-expression of HBXIP suppressed apoptosis in collaboration with Survivin.

Detail Description Paragraph - DETX (203):
[0191] It is estimated that more than 380 million HBV carriers are present worldwide today, with chronic HBV infection representing a major global pathogenic factor for development of hepatocellular carcinoma (Lok, A. S. 2000 J Hepatol 32:89-97). A crucial role of HBV in hepatocarcinogenesis is beyond doubt, while the mechanisms by which HBV causes the transformation of hepatocytes remain unclear. The HBV genome consists in a 3.2 kbp circular double-stranded DNA molecule with overlapping open reading frames (ORFs) encoding four viral proteins. Among them is HBX, a 154 amino-acid protein that has no recognizable counterpart in humans or other mammalian species. HBX is essential for replication of woodchuck HBV, and transgenic mice engineered to express HBX have increased incidence of hepatocellular cancer, especially when exposed to chemical carcinogens (Kim, C. M. et al. 1991 Nature 351:317-320). Thus, HBX is a candidate transforming gene of HBV. Like many viral oncoproteins, HBX is multifunctional protein. HBX exhibits effects on gene transcription, cell proliferation, and apoptosis, and has multiple putative cellular targets besides HBXIP (reviewed in Murakami, S. 2001 J Gastroenterol 36:651-660). The effects of HBX on apoptosis are controversial, with evidence suggestive either of suppression or promotion of apoptosis, depending on the cellular context and stimulus. Among the reported molecular effects of HBX is transcription-independent suppression of Caspases (Gottlob, K. et al. 1998 J Biol Chem 273:33347-33353), though many alternative mechanisms have also been proposed (reviewed in Murakami, S. 2001 J Gastroenterol 36:651-660). These data demonstrate that HBX can associate indirectly with Survivin, through HBXIP. Moreover, depletion of Survivin from cell extracts abolishes the ability of HBX to suppress Caspase activation in vitro. Thus, HBX modulates

apoptosis pathways through interactions with HBXIP/Survivin complexes.

Claims Text - CLTX (9):
8. A method for treating human liver disease associated with HBV, comprising administration of an inhibitor of HBXIP in the presence of Survivin.

Claims Text - CLTX (17):
16. A method for identifying an agent that effectively inhibits the activity of HBX, comprising: contacting HBX with a cell extract containing Survivin and pro-Caspase-9 in the presence of a compound; inducing activation of pro-Caspase-9; and measuring the activation of pro-Caspase-9, thereby determining whether said compound is an agent that effectively inhibits the activity of HBX.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040106539 A1

TITLE: Agents for the treatment of viral infections

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APPL-NO: 10/ 398993

DATE FILED: October 30, 2003

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DE	100 51 716.1	2000DE-100 51 716.1	October 12, 2000
DE	101 49 398.3	2001DE-101 49 398.3	October 3, 2001

PCT-DATA:
APPL-NO: PCT/DE01/03908
DATE-FILED: Oct 11, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 514/2

ABSTRACT:

The invention relates to agents for the treatment of viral infections, in particular, infections with hepatitis and retro-viruses. Said agents inhibit the release, maturation and replication of both retro-viruses and also hepatitis viruses. In the example of human immune deficiency virus (HIV) and hepatitis-B viruses it has been shown that proteasome inhibitors block the release of virus particles and the infectiousness of the released viral particles and thus the reproduction of the viruses. The proteasome inhibitors affect the activities in the ubiquitin/proteasome pathway, in particular the enzymatic activities of the 26S and the 20S proteasome complexes. The application for the above invention lies in anti-retroviral therapy, particularly the treatment of HIV infections and AIDS and in the anti-viral therapy of hepatitis infections, in particular the treatment of acute and chronic HBV and HCV infections and the associated liver carcinomas.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (2):
[0002] Furthermore, said compositions may be used for the treatment, therapy and inhibition of a viral hepatitis. It is demonstrated that the applications of said compositions result in the release of noninfectious hepatitis viruses from infected cells. Said compositions may therefore limit the spread of an acute infection with hepatitis viruses. Furthermore, the compositions are less toxic for nonproliferating hepatocytes than for nonparenchymal liver cells and liver carcinoma cells. Thus, said compositions are suitable for a preferred destruction of liver carcinoma cells in patients and animals infected with HBV (hepatitis B viruses, list of abbreviations after the examples) and HCV (hepatitis C viruses). The compositions are various substance classes which share the ability to inhibit the ubiquitin-proteasome system. Specifically, said compositions are characterized in that said substances, the proteasome inhibitors, inhibit the activity of the major cellular protease, i.e. the proteasome, in the treated cells. On the basis of the example of the duck hepatitis virus as well as the human hepatitis B virus, it is demonstrated that the application of proteasome inhibitors drastically reduces the release of infectious duck hepatitis viruses and human hepatitis B viruses from already infected hepatocytes (demonstrated, by way of example, for the duck hepatitits virus). Said proteasome inhibitors can therefore suppress viremia in the case of both a new infection and chronic infections with hepatitis viruses and successful virus elimination by the endogenous immune system and/or by known compositions having a similar or different action can be enhanced. Using said compositions, namely the proteasome inhibitors, can prevent, reduce or reverse the consequences of a HBV and HCV infection, such as, for example, liver damage of differing degrees of severity up to the frequently fatal fulminant hepatitis, development of liver cirrhosis/fibrosis and of liver carcinoma. Fields of application for these inventions are therefore antiretroviral therapy and the prevention of infections with immunodeficiency-causing antiviruses, especially the acquired immunodeficiency in animals and humans, in particular of HIV-1/HIV-2 infections and AIDS, as well as antiviral therapy of hepatitis virus infections, in particular for preventing acute and chronic HBV and HCV infections from being established and maintained.

Summary of Invention Paragraph - BSTX (59):
[0058] The nucleoside analogs used for HBV inhibit transcription of the pregenomic RNA into DNA by the virus-encoded polymerase. Nucleoside analogs (e.g. lamivudine, famcyclovir, adevofir and entacavir) have the great disadvantage of almost always causing the selection of medicament-resistant HBV strains. Moreover, nucleoside analogs harbor the risk of possibly causing chromosomal mutations and thus cancer. The novel medicaments mentioned within the scope of the invention presented herein do not carry this risk and said side effects.

Summary of Invention Paragraph - BSTX (63):
[0062] One of the regulatory proteins of HBV, the HBx protein, was shown to interact with a subunit of the 26S proteasome complex, said interaction being essential for HBx function (Hu et al., 1999). It was furthermore reported that HBV- and HCV-antigenic determinants can be efficiently presented in the form of MHC class I peptide complexes even in the presence of proteasome inhibitors (Lpez et al., 2000). Thus, a therapy with proteasome inhibitors, as proposed in the present specification, would not influence the recognition of HBV-infected liver cells by cytotoxic T.sub.CD8+ cells and thus the cellular natural immunity against HBV infection.

Summary of Invention Paragraph - BSTX (68):
[0067] On the basis of the known prior art, it can be stated that the

surprisingly found antiviral action of proteasome inhibitors on late processes of retroviral replication, such as, for example, Gag processing, assembling and budding of HIV-1 or HIV-2 virions, and on the production of infectious virus progeny or on the entire virus replication cycle has not been reported as yet. Likewise, there are no reports on the use of proteasome inhibitors for the treatment of infections with HIV or other retroviruses. Furthermore, it can be concluded that none of the studies previously published in the specialist or patent literature or other work published to date have tested or reported an influence exerted by proteasome inhibitors on the release and infectivity of hepatitis viruses, as will be illustrated in the present description of the invention. In particular, no antiviral effects by proteasome inhibitors on the processes of the HBV replication cycle have been determined. Furthermore, it has not been reported that proteasome inhibitors preferably destroy liver carcinoma cells generated by hepatitis infections and are therefore suitable for the therapy of liver carcinomas. The actions of proteasome inhibitors, illustrated according to the invention, on early and late processes of HBV replication and also on the development of secondary liver cirrhosis and liver carcinomas thus represent entirely novel principles of the antiviral treatment of HBV infections. The use of proteasome inhibitors in the antiviral therapy of hepatitis infections, especially for preventing an acute and chronic HBV and HCV infection from being established or maintained, has not been reported to date.

Summary of Invention Paragraph - BSTX (164):

[0162] In another part, the second main focus of the invention, it is surprisingly found that, similarly to the effect on retroviruses, proteasome inhibitors inhibit late processes in the replication cycle of hepadnaviruses. In this connection, it was specifically observed that the inventive use of proteasome inhibitors is suitable for preventing substantially or completely the production of infectious virions from chronically HBV-infected cells. Treatment of HBV-producing cells with proteasome inhibitors entails both inhibition of the release of virions and a virtually complete reduction in the infectivity of the released virions. As a consequence of these novel activities, proteasome inhibitors can suppress virus replication and thus de novo infection of hepatocytes and thus the spread of an HBV infection in vivo in the liver tissue of an HBV-infected individual.

Summary of Invention Paragraph - BSTX (169):

[0167] Another use of proteasome inhibitors is prevention of a hepatitis virus infection in individuals at high risk of a new infection, such as, for example, doctors, high-risk personnel in buildings with large numbers of visitors, drug addicts, travelers in regions in which hepatitis viruses are highly endemic, in the treatment of patients, for relatives of chronic virus carriers. Another use of proteasome inhibitors is prevention of reinfection with HBV in the case of liver and other organ transplants and in the case of cell therapies by administering the compositions prior to, during and after transplantation. The administration of said compositions is indicated both for the high-risk situation when transplanting virus-free organs to chronic virus carriers who continuously have residual viruses which can infect the new organs, and for transferring virus-containing organs from donors to virus-free patients.

Summary of Invention Paragraph - BSTX (189):

[0187] In another embodiment of the invention it is demonstrated that treatment of chronically HBV-infected human hepatoma cell lines with proteasome inhibitors causes the release of HBV-specific proteins, in particular of HBs and HBe antigen (for this, see exemplary embodiment 10). Similarly to the action of proteasome inhibitors on DHBV, a distinct inhibition of the secretion of HBV proteins was observed.

Summary of Invention Paragraph - BSTX (190):

[0188] In another form of the invention it is demonstrated that, owing to the novel action on the release and infectivity of hepatitis viruses, proteasome inhibitors prevent the spread of an HBV infection in cultured hepatocytes. According to the invention, it is detected that it is completely prevented by the secondary infection, i.e. the transfer of an already established infection to neighboring cells. This inhibitory effect on the secondary infection was detected in primary duck hepatocytes which, after primary infection, were treated with proteasome inhibitors for several days. According to the novel effects of proteasome inhibitors, both a lower expression of the viral antigens and, due to blockage of the secondary infection, a lower number of de novo infected cells were found in the primarily infected cells treated (for this, see exemplary embodiment 8).

Summary of Invention Paragraph - BSTX (204):

[0202] Within the scope of the invention, it is found for the first time that proteasome inhibitors both inhibit maintenance and persistence of an already established infection and completely block in hepatocytes the secondary infection and thus the spread of an infection with hepatitis viruses in vivo. According to the present invention, proteasome inhibitors are substances suitable for blocking the spread of an HBV infection in vivo.

Summary of Invention Paragraph - BSTX (206):

[0204] Owing to this novel method of treatment, it is therefore possible to produce a multiplicity of therapeutic effects by using proteasome inhibitors in infections with hepadnaviruses. In addition to blocking the infectivity of the released viruses and preventing liver cell carcinomas, said method of treatment has another advantage in that this strategy affects cellular factors which are essential for the replication of hepadnaviruses but which comprise a very much higher genetic stability compared to viral factors. Owing to said genetic stability of the target structure of this novel antiviral strategy, the appearance of resistance symptoms, as known for many of the previously known inhibitors of an HBV infection, is not a factor. This applies, in particular, to the polymerase mutants of hepatitis B and C viruses, which, in the case of nucleoside analog treatment, practically always appear after a relatively short time. This applies also to immunoescape variants as appearing in passively and actively vaccinated patients as well as naturally. The same applies to interferon-resistant strains of HBV and HCV. This novel effect of proteasome inhibitors is the basis of the inventive claim that treatment with proteasome inhibitors makes it possible to not only prevent the spread of an HBV infection but also to treat liver cell carcinomas caused by HBV.

Summary of Invention Paragraph - BSTX (272):

[0253] Overall, these experiments demonstrate that proteasome inhibitors inhibit both the establishment and the persistence of a primary infection. In addition, they prevent the spread of the primary DHBV infection by blocking the progeny viruses. As a result, proteasome inhibitors are substances suitable for blocking the spread of an HBV infection in vivo.

Summary of Invention Paragraph - BSTX (287):

[0264] Effect of Proteasome Inhibitors on the Secretion of HBV-Specific Antigens in Human Hepatoma Cell Lines

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DOCUMENT-IDENTIFIER: US 20040029110 A1

TITLE: Method for typing and detecting HBV

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INVENTOR-INFORMATION:

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COUNTRY	APPL-NO	DOC-ID	APPL-DATE
EP	96870053.4	1996EP-96870053.4	April 19, 1996

US-CL-CURRENT: 435/5, 435/6

ABSTRACT:

The present invention relates to a method for detection and/or genetic analysis of HBV in a biological sample, comprising hybridizing the polynucleic acids of the sample with a combination of at least two nucleotide probes, with said combination hybridizing specifically to a mutant target sequence chosen from the HBV RT pol gene region and/or to a mutant target sequence chosen from the HBV preCore region and/or to a mutant target sequence chosen from the HBsAg region of HBV and/or to a HBV genotype-specific target sequence, with said target sequences being chosen from FIG. 1, and with said probes being applied to known locations on a solid support and with said probes being capable of hybridizing to the polynucleic acids of the sample under the same hybridization and wash conditions, or with said probes hybridizing specifically with a sequence complementary to any of said target sequences, or a sequence wherein T of said target sequence is replaced by U; and detecting the hybrids formed; and inferring the HBV genotype and/or mutants present in said sample from the differential hybridization signal(s) obtained. The invention further relates to sets of nucleotide probes and possibly primers useful in said methods as well as to their use in a method for typing and/or detecting HBV and to assay kits using the same.

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Summary of Invention Paragraph - BSTX (6):
[0006] Lamivudine is a (-) enantiomer of 3' thiacytidine, a 2'3'-dideoxynucleoside analogue, and is known to be a potent inhibitor of HBV replication through inhibition of the reverse transcriptase (RT) activity of the HBV polymerase. Lamivudine treatment can result in histological improvements in chronic hepatitis patients, and when given pre- and post-liver transplantation, it can prevent graft reinfection (Honkoop et al., 1995; Naoumov et al., 1995). However, after treatment, a hepatitis flare-up can be observed in most patients, with ALT elevations and HBV DNA that becomes detectable again. This HBV DNA rebound is associated with a new quasi species equilibrium. In a few cases, virus breakthrough during therapy was observed, due to the selection of lamivudine resistent HBV strains. The exact nature of this breakthrough has been ascribed to the accumulation of mutations in the RT part of the Polymerase. A similar mechanism in the HIV RT polymerase has been found, where upon lamivudine treatment, mutations accumulate in the YMDD motif (Gao et al., 1993). This YMDD motif is also present in the RT part of the HBV polymerase, and lamivudine-selected mutations in HBV have been found in this region (Tipples et al., 1996), as well as in other regions of the RT part of the polymerase (Ling et al., 1995). Penciclovir is another drug that has been shown to inhibit the reverse transcriptase activity of the HBV polymerase (Shaw et al., 1996), and mutations in the HBV polymerase may also be detected upon treatment with this drug.

Brief Description of Drawings Paragraph - DRTX (2):
[0153] FIG. 1: Alignment of 35 complete HBV genomes. Isolates belonging to genotype A are: HBVXCPS, HBVADW, HVHEPB, S50225, HPBADWZCG; genotype B: HPBADW3, HPBADWZ, HPBADW1, HPBADW2; genotype C: HPBCGADR, HBVADRM, HPBADRA, HPBCG, HEHBVAYR, HBVADR, HBVADR4, HPBADR1C, HPBADRC, HBVPREX, HPBETNC, HHVBC, HHVCCHA; genotype D: HBVAYWMCG, HBVAYWC, HBVAYWCI, HBVAYWE, HBVDNA, HPBHBVAA, HBVADW4A. To preserve alignment, several gaps were created in the alignment and are indicated with /. Positions of start and end of the different HBV encoded genes is indicated: HBsAg: hepatitis B surface antigen (small surface antigen); HBx: hepatitis B X protein; HB Pol: hepatits B polymerase protein, encoding a terminal protein, a spacer, a RT/DNA polymerase region, and an RNAse H activity; HBcAg: hepatitis B Core antigen; HBpreS1Ag: hepatitis B preS1 antigen (large surface antigen); HBpreS2Ag: hepatitis B preS2 antigen (middle surface antigen). The position of the PCR primers is indicated with a large box over all 35 sequences. The polarity of the PCR primer can be deduced from the position of the name above these boxes: left=antisense primer; right=sense primer. LiPA probes are indicated with small boxes, the numbers of the probes are indicated next to the probes or to the right of the alignment, and correspond to the probe numbers in Table 1.

Detail Description Paragraph - DETX (77):
[0218] Shaw, T., Mok, S. S., Locarnini, S. A. Inhibition of hepatitis B virus DNA polymerase by enantiomers of penciclovir triphosphate and metabolic basis for selective inhibition ox HBV replication by penciclovir. Hepatology 1996; 24: 996-1002.

Detail Description Table CWU - DETL (1):
1TABLE 1 HBV probe and primer design Name Sequence SEQ ID NO Region HBPr1 GGGTCACCATATTCTTGGG 1 preS1 primer sense HBPr2 GAACAAGAGCTACAGCATGGG 2 preS1 primer sense HBPr3 CCACTGCATGGCCTGAGGATG 3 preS1 primer anti-sense HBPr4 GTTCCT/GGAACTGGAGCCACCAG 4 preS1 primer anti-sense HBPr5 ICOOOGTATTAGGAGGCTOTAG 5 preS1 primer sense HBPr6 GCTGTAGGCATAAATTGGICTG 6 preS1 primer sense HBPr7 CTCCAGGCATAAAIIGGICTG 7 preS1 primer anti-sense

HBPr8 GAAGGAAAGAAGTCAGAAGGC 8 preS1 primer anti-sense HBPr9 TGGCTTTGGGGCATGG 9 preCore HBPr10 TGGCTTTAGGGCATGG 10 preCore HBPr11 TGGCTTAGGACATGG 11 preCore HBPr12 AAGTTGCATGGIGCTG 12 preCore HBPr13 CACCTCTGCCTAATCAT 13 preCore HBPr14 TGGGGTGGAGCCCTCAG 14 preS1 HBPr15 GCCAGCAGCCAACCAG 15 preS1 HBPr16 CCCATGGGGGACTGT 16 preS1 HBPr17 AACCCCAACAAGGATG 17 preS1 HBPr18 TCCACCAGCAATCCT 18 preS1 HBPr19 TGGGGGAAGAATATTT 19 preS1 HBPr20 AAATTCCAGCAGTCCC 20 preS1 HBPr21 GTTCCCAACCCTCTGG 21 preS1 HBPr22 AACCTCGCAAAGGCAT 22 preS1 HBPr23 TGCATTCAAAGCCAAC 23 preS1 HBPr24 TACTCACAACTGTGCC 24 preS1 HBPr25 ACCCTGCGTTCGGAGC 25 preS1 HBPr26 CAGGAAGACAGCCTAC 26 preS1 HBPr27 GATCCAGCCTTCAGAG 27 preS1 HBPr28 ATGCTCCAGCTCCTAC 28 preS1 HBPr29 GCTTTCTTGGACGGTC 29 preS1 HBPr30 CTACCCCAATCACTCC 30 preS1 HBPr31 AGCACCTCTCTCAACG 31 preS1 HBPr32 CCAATGGCAAACAAGG 32 preS1 HBPr33 CTGAGGGCTCCACCCCA 33 preS1 HBPr34 ATGCAACTTTTTCACC 34 preCore HBPr35 ATCTCTTGTACATGTC 35 preCore HBPr36 ATCTCATGTTCATGTC 36 preCore HBPr37 CAGTGGGACATGTACA 37 preCore HBPr38 CAGTAGGACATGAACA 38 preCore HBPr39 CTGTTCAAGCCTCCAA 39 preCore HBPr40 AGCCTCCAAGCTGTGC 40 preCore HBPr41 AAAGCCACCCAAGGCA 41 preCore HBPr42 TGGCTTTAGGACATGGA 42 preCore HBPr43 GACATGTACAAGAGATGA 43 preCore HBPr44 GACATGAACATGAGATGA 44 preCore HBPr45 TGTACATGTCCCACTGTT 45 preCore HBPr46 TGTTCATGTCCTACTGTT 46 preCore HBPr47 ACTGTTCAAGCCTCCAAG 47 preCore HBPr48 GGCACAGGCTTGGAGGCTT 48 preCore HBPr49 AAAGCCACCCAAGGCACA 49 preCore HBPr50 CCCAGAGGGTTGGGAAC 50 preS1 HBPr51 CAGCATGGGGCAGAATCT 51 preS1 HBPr52 TCCACCAGCAATCCTCTG 52 preS1 HBPr53 GGATCCAGCCTTCAGAGC 53 preS1 HBPr54 TCAGGAAGACAGCCTAC 54 preS1 HBPr55 TTCAACCCCAACAAGGATC 55 preS1 HBPr56 AATGCTCCAGCTCCTAC 56 preS1 HBPr57 CTGCATTCAAAGCCAACT 57 preS1 HBPr58 CCCCATGGGGGACTGTTG 58 preS1 HBPr59 CATACTCACAACTGTGCCA 59 preS1 HBPr60 GGGCTTTCTTGGACGGTCC 60 preS1 HBPr61 CTCTCGAATGGGGGAAGA 61 preS1 HBPr62 CCTACCCCAATCACTCCA 62 preS1 HBPr63 AGCACCTCTCTCAACGACA 63 preS1 HBPr64 GCAAATTCCAGCAGTCCCG 64 preS1 HBPr65 GCCAATGGCAAACAAGGTA 65 preS1 HBPr66 GACATGAACATGAGATG 66 preCore HBPr67 GGACATGAACAAGAGAT 67 preCore HBPr68 GACATGTACAAGAGATG 68 preCore HBPr69 ACATAAGAGGACTCTTGGAC 69 preCore primer sense HBPr70 TACTTCAAAGACTGTGTGTTTA 70 preCore primer sense HBPr71 ACAAAGACCTTTAAC/TCT 71 preCore promoter HBPr72 ACAAAGATCATTAAC/TCT 72 preCore promoter HBPr73 TTCCACCAGCAATCCTC 73 preS1 HBPr74 GATCCAGCCTTCAGAGC 74 preS1 HBPr75 CAAGGTATGTTGCCCGTTTGTCC 75 HBsAg primer sense HBPr76 CCAAACAGTGGGGGAAAGCCC 76 HBsAg primer anti-sense HBPr77 CTACGGATGGAAATTGC 77 HBsAg codon 145 wild type HBPr78 TACGGACGGAAACTGC 78 HBsAg codon 145 wild type HBPr79 TTCGGACGGAAACTGC 79 HBsAg codon 145 wild type HBPr80 CTTCGGACGGAAATTGC 80 HBsAg codon 145 wild type HBPr81 CTACGGATAGAAATTGC 81 HBsAg codon 145 mutant HBPr82 CTTCGGACAGAAATTGC 82 HBsAg codon 145 mutant HBPr83 CTATGGGAGTGGGCCTCAGT/CC 83 HB Pol HBPr84 GCTGTAGGCATAAATTGGTCTG 84 preCore primer sense HBPr85 CTCCACAGT/AAGCTCCAAATTC 85 preCore primer anti-sense HBPr86 ACATAAGAGGACTCTTGGAC 86 preCore primer sense HBPr87 TACTTCAAAGACTGTGTGTTTA 87 preCore primer sense HBPr88 TAGGTTAAAGGTCTTTGT 88 preCore promoter HBPr89 TAGGTTAATGATCTTTGT 89 preCore promoter HBPr90 CATGTCCCACTGTTCAA 90 preCore HBPr91 CATGTCCTACTGTTCAA 91 preCore HBPr92 TTCTGCCCCATGCTGTA 92 preS1 HBPr93 TTCTGCCCCATGCTGTAG 93 preS1 HBPr94 GGTAA/TAAAGGGACTCAC/AGATG 94 HBsAg primer anti-sense HBPr95 TCAGCTATATGGATGAT 95 HB Pol HBPr96 CAGCTATATGGATGAT 96 HB Pol HBPr97 TTCAGCTATATGGATG 97 HB Pol HBPr98 TCAGTTATATGGATGAT 98 HB Pol HBPr99 TTTCAGTTATATGGATG 99 HB Pol HBPr100 TTTAGTTATATGGATGA 100 HB Pol HBPr101 TCAGCTATGTGGATGAT 101 HB Pol HBPr102 TCAGTTATGTGGATGAT 102 HB Pol HBPr103 TTTCAGCTATGTGGATG 103 HB Pol HBPr104 CAAGGTATGTTGCCCGTTTGTCC 104 HBsAg primer sense HBPr105 GGT/CAA/TAAAGGGACTCAC/AGATG 105 HBsAg primer anti-sense HBPr106 GGGTCACCATATTCTTGGG 106 preS1 primer sense HBPr107 GTTCCT/GGAACTGGAGCCACCAG 107 preS1 primer anti-sense HBPr108 CCGGAAAGCTTGAGCTCTTCTTTTTCACCTCTGCCTAATC 108 preCore primer sense HBPr109 CCGGAAAGCTTGAGCTCTTCAAAAAGTTGCATGGTGCTGG 109 preCore primer anti-sense HBPr110 CCTCTGCCGATCCATACTGCGGAAC 110 preX primer sense HBPr111 CTGCGAGGCGAGGGAGTTCTTCTTC 111 HB Core primer anti-sense

HBPr112 TGCCATTTGTTCAGTGGTTCGTAGGGC 112 HBsAg primer sense HBPr113 CCGGCAGATGAGAAGGCACAGACGG 113 HBX primer antisense HBPr114 TTCAGCTATATGGATGAT 114 YMDD motif HBPr115 TCAGCTATATGGATGATG 115 YMDD motif HBPr116 TTCAGCTATGTGGATGAT 116 YMDD motif HBPr117 TCAGCTATGTGGATGATG 117 YMDD motif HBPr118 GGCTTTGGGGCATGG 118 preCore codon 28 wild type HBPr119 TGGCTTTGGGGCATG 119 preCore codon 28 wild type HBPr120 GTGGCTTTGGGGCATG 120 preCore codon 28 wild type HBPr121 GGCTTTGGGGCATGGA 121 preCore codon 28 wild type HBPr122 TGGCTTTGGGACATGG 122 preCore codon 28 wild type, codon 29 mutant HBPr123 GGCTTTGGGACATGG 123 preCore codon 28 wild type, codon 29 mutant HBPr124 TGGCTTTGGGACATG 124 preCore codon 28 wild type, codon 29 mutant HBPr125 GTGGCTTTGGGACATG 125 preCore codon 28 wild type, codon 29 mutant HBPr126 GGCTTTGGGACATGGA 126 preCore codon 28 wild type, codon 29 mutant HBPr127 TCAGTTATATGGATGATG 127 YMDD genotype D, wild tpye HBPr128 TTCAGTTATATGGATGAT 128 YMDD genotype D, wild tpye HBPr129 TTTCAGTTATATGGATGAT 129 YMDD genotype D, wild tpye HBPr130 TCAGTTATGTGGATGATG 130 YMDD genotype D, mutant HBPr131 TTCAGTTATGTGGATGAT 131 YMDD genotype D, mutant HBPr132 TTTCAGTTATGTGGATGAT 132 YMDD genotype D, mutant HBPr133 TTTCAGTTATGTGGATGA 133 YMDD genotype D, mutant HBPr134 TGCTGCTATGCCTCATCTTC 134 outer HBsAg primer sense HBPr135 CA(G/A)AGACAAAAGAAAATTGG 135 outer HBsAg primer anti-sense HBPr136 CTATGGATGGAAATTGC 136 HBsAg mutant codon 143 HBPr137 CCTATGGATGGAAATTG 137 HBsAg mutant codon 143 HBPr138 ACCTATGGATGGAAATT 138 HBsAg mutant codon 143 HBPr139 CT CAA GGC AAC TCT ATG TGG 139 HBsAg, genotype A HBPr140 CT CAA GGC AAC TCT ATG GG 140 HBsAg, genotype A HBPr141 T CAA GGC AAC TCT ATG TTG 141 HBsAg, genotype A HBPr142 ATC CCA TCA TCT TGG G 142 HBsAg, genotype B HBPr143 ATC CCA TCA TCT TGG GCG G 143 HBsAg, genotype B HBPr144 TC CCA TCA TCT TGG GCG G 144 HBsAg, genotype B HBPr145 C CCA TCA TCT TGG GCT GG 145 HBsAg, genotype B HBPr146 TTC GCA AAA TAC CTA TGG 146 HBsAg, genotype B HBPr147 T TTC GCA AAA TAC CTA TG 147 HBsAg, genotype B HBPr148 CT TTC GCA AAA TAC CTA TG 148 HBsAg, genotype B HBPr149 TC GCA AAA TAC CTA TGG G 149 HBsAg, genotype B HBPr150 T CTA CTT CCA GGA ACA T 150 HBsAg, genotype C HBPr151 T CTA CTT CCA GGA ACA TC 151 HBsAg, genotype C HBPr152 CT CTA CTT CCA GGA ACA T 152 HBsAg, genotype C HBPr153 CT CTA CTT CCA GGA ACA G 153 HBsAg, genotype C HBPr154 C TGC ACG ATT CCT GCT 154 HBsAg, genotype C HBPr155 TGC ACG ATT CCT GCT CA 155 HBsAg, genotype C HBPr156 C TGC ACG ATT CCT GCT C 156 HBsAg, genotype C HBPr157 TGC ACG ATT CCT GCT CAA 157 HBsAg, genotype C HBPr158 TTC GCA AGA TTC CTA TG 158 HBsAg, genotype C HBPr159 CT TTC GCA AGA TTC CTA T 159 HBsAg, genotype C HBPr160 CT TTC GCA AGA TTC CTA 160 HBsAg, genotype C

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INVENTOR-INFORMATION:

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non-provisional-of-provisional 60057254 19970829 US

US-CL-CURRENT: 514/44, 424/85.4 , 424/93.2

ABSTRACT:

Methods and pharmaceutical compositions for modifying cells of a mammalian recipient with DNA encoding a secreted protein such as human interferon in situ are provided. The methods include forming a secreted protein expression system in vivo or ex vivo and administering the expression system to the mammalian recipient. The expression system and methods are useful for the localized and systemic delivery of interferons in situ.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (7):
[0005] Interferons also appear to have antiviral activities that are based on two different mechanisms. For instance, type I interferon proteins (.alpha. and .beta.) can directly inhibit the replication of human hepatitis B virus ("HBV") and hepatitis C virus ("HCV"), but can also stimulate an immune response which attacks cells infected with these viruses.

Detail Description Paragraph - DETX (94):
[0118] It is also possible to design a vector in which the interferon gene is only expressed in hepatitis-infected cells. For instance, expression of the interferon gene could be induced by the HBV transcription factor HBx which will only be present in HBV-infected hepatocytes. In addition, a "carrier" system

may be used. This could be a non-viral delivery system such as cationic liposomes or protein:DNA conjugates (conjugates of DNA with asialoglycoproteins can be delivered preferentially to the liver via binding to the asialoglycoprotein receptor on hepatocytes).

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DOCUMENT-IDENTIFIER: US 20030206887 A1

TITLE: RNA interference mediated inhibition of hepatitis B virus (HBV) using short interfering nucleic acid (siNA)

PUBLICATION-DATE: November 6, 2003

INVENTOR-INFORMATION:

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child 09877478 20010608 US

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child 09636385 20000809 US

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US-CL-CURRENT: 424/93.2, 514/44 , 536/23.1

ABSTRACT:

The present invention concerns methods and reagents useful in modulating hepatitis B virus (HBV) gene expression in a variety of applications, including use in therapeutic, diagnostic, target validation, and genomic discovery applications. Specifically, the invention relates to short interfering nucleic acid (siNA) or short interfering RNA (siRNA) molecules capable of mediating RNA interference (RNAi) against against hepatitis B virus (HBV).

PRIORITY

[0001] This application claims the benefit of U.S. application Ser. Nos. 60/358,580, filed Feb. 20, 2002, and 60/393,924, filed Jul. 3, 2002. This application also claims priority to PCT US02/09187, filed Mar. 26, 2002, which claims the benefit of U.S. application Ser. No. 60/296,876, filed Jun. 8, 2001.

---------- KWIC ---------

Title - TTL (1):
RNA interference mediated inhibition of hepatitis B virus (HBV) using short interfering nucleic acid (siNA)

Summary of Invention Paragraph - BSTX (37):
[0037] Lamivudine (3TC.RTM.) is a nucleoside analogue, which is a very potent and specific inhibitor of HBV DNA synthesis, and has recently been approved for the treatment of chronic hepatitis B. Unlike treatment with interferon, treatment with 3TC.RTM. does not eliminate the HBV from the patient. Rather, viral replication is controlled and chronic administration results in improvements in liver histology in over 50% of patients. Phase III studies with 3TC.RTM., showed that treatment for one year was associated with reduced liver inflammation and a delay in scarring of the liver. In addition, patients treated with 3TC.RTM. (100 mg per day) had a 98% reduction in hepatitis B DNA and a significantly higher rate of seroconversion, suggesting disease improvements after completion of therapy. However, cessation of therapy resulted in a reactivation of HBV replication in most patients. In addition, recent reports have documented 3TC.RTM. resistance in approximately 30% of patients.

Summary of Invention Paragraph - BSTX (47):

[0046] In one embodiment, a siNA molecule of the invention has RNAi activity that modulates expression of RNA encoded by HBV genes, for example genes required for viral replication including genes required for HBV protein synthesis, such as the 5'-most 1500 nucleotides of the HBV pregenomic mRNA. This region controls the translational expression of the core protein (C), X protein (X), and DNA polymerase (P) genes, and plays a role in the replication of the viral DNA by serving as a template for reverse transcriptase. Disruption of this region in the RNA results in deficient protein synthesis as well as incomplete DNA synthesis (and inhibition of transcription from the defective genomes). Target sequences 5' of the encapsidation site can result in the inclusion of the disrupted 3' RNA within the core virion structure, and targeting sequences 3' of the encapsidation site can result in the reduction in protein expression from both the 3' and 5' fragments. Alternative regions outside of the 5'-most 1500 nucleotides of the pregenomic mRNA also make suitable targets of siNA-mediated inhibition of HBV replication. Such targets include the mRNA regions that encode the viral S gene. Selection of particular target regions will depend upon the secondary structure of the pregenomic mRNA. Targets in the minor mRNAs can also be used, especially when folding or accessibility assays in these other RNAs reveal additional target sequences that are unavailable in the pregenomic mRNA species. A desirable target in the pregenomic RNA is a proposed bipartite stem-loop structure in the 3'-end of the pregenomic RNA that is believed to be critical for viral replication (Kidd and Kidd-Ljunggren, 1996. Nuc. Acid Res. 24:3295-3302). The 5'-end of the HBV pregenomic RNA carries a cis-acting encapsidation signal, which has inverted repeat sequences that are thought to form a bipartite stem-loop structure. Due to a terminal redundancy in the pregenomic RNA, the putative stem-loop also occurs at the 3'-end. While it is the 5' copy that functions in polymerase binding and encapsidation, reverse transcription actually begins from the 3' stem-loop. To start reverse transcription, a 4 nucleotide primer that is covalently attached to the polymerase is made, using a bulge in the 5' encapsidation signal as template. This primer is then shifted, by an unknown mechanism, to the DRI primer binding site in the 3' stem-loop structure, and reverse transcription proceeds from that point. The 3' stem-loop, and especially the DRI primer binding site, appear to be highly effective targets for siNA mediated intervention. Sequences of the pregenomic RNA are shared by the mRNAs for surface, core, polymerase, and X proteins. Due to the overlapping nature of the HBV transcripts, all share a common 3'-end. Therefore, siNA targeting of this common 3'-end will thus disrupt the pregenomic RNA as well as all of the mRNAs for surface, core, polymerase and X proteins.

Summary of Invention Paragraph - BSTX (49):

[0048] In one embodiment, the invention features one or more siNA molecules and methods that independently or in combination modulate the expression of genes representing cellular targets for HBV infection, such as cellular receptors, cell surface molecules, cellular enzymes, cellular transcription factors, and/or cytokines, second messengers, and cellular accessory molecules including but not limited to interferon regulatory factors (IRFs such as Genbank Accession No. AF082503.1), cellular PKR protein kinase (such as Genbank Accession No. XM.sub.--002661.7), human eukaryotic initiation factors 2B (eIF2Bgamma, such as Genbank Accession No. AF256223 and/or eIF2gamma, such as Genbank Accession No. NM.sub.--006874.1), human DEAD Box protein DDX3 (such as Genbank Accession No. XM.sub.--018021.2), and cellular proteins that are essential for the maintenance of persistent infection of hepatocytes, such as proteins that interact with the HBV-encoded HBx regulatory protein.

Summary of Invention Paragraph - BSTX (111):

[0110] The siNA molecules of the invention can be designed to inhibit target

(HBV) gene expression through RNAi targeting of a variety of RNA molecules. In one embodiment, the siNA molecules of the invention are used to target various RNAs corresponding to a target gene. Non-limiting examples of such RNAs include messenger RNA (mRNA), alternate RNA splice variants of target gene(s), post-transcriptionally modified RNA of target gene(s), pre-mRNA of target gene(s), and/or RNA templates. If alternate splicing produces a family of transcipts that are distinguished by usage of appropriate exons, the instant invention can be used to inhibit gene expression through the appropriate exons to specifically inhibit or to distinguish among the functions of gene family members. For example, a protein that contains an alternatively spliced transmembrane domain can be expressed in both membrane bound and secreted forms. Use of the invention to target the exon containing the transmembrane domain can be used to determine the functional consequences of pharmaceutical targeting of membrane bound as opposed to the secreted form of the protein. Non-limiting examples of applications of the invention relating to targeting these RNA molecules include therapeutic pharmaceutical applications, pharmaceutical discovery applications, molecular diagnostic and gene function applications, and gene mapping, for example using single nucleotide polymorphism mapping with siNA molecules of the invention. Such applications can be implemented using known gene sequences or from partial sequences available from an expressed sequence tag (EST).

Brief Description of Drawings Paragraph - DRTX (30):

[0202] FIG. 11 shows a graphical representation of siNA mediated inhibition of HBV in a cell culture experiment. Results are shown with reference to the siRNA construct used (sense strand SEQ ID NO: 1338/antisense strand SEQ ID NO: 1342) at different lipid concentrations (2.5, 5.0, 7.5, 10.0 and 12.5 ug/ml). Inverted sequence duplexes were used as negative controls (sense strand SEQ ID NO: 1358/antisense strand SEQ ID NO: 1350). Levels of secreted HBV surface antigen (HBsAg) were analyzed by ELISA.

Detail Description Paragraph - DETX (104):

[0298] In an alternate approach, a pool of siNA constructs specific to an HBV target sequence is used to screen for target sites in cells expressing HBV RNA. The general strategy used in this approach is shown in FIG. 9. Cells expressing HBV (e.g., HEPG2) are transfected with the pool of siNA constructs and cells that demonstrate a phenotype associated with HBV inhibition are sorted. The pool of siNA constructs can be expressed from transcription cassettes inserted into appropriate vectors (see for example FIG. 7 and FIG. 8). Cells in which HBV expression is decreased due to siNA treatment demonstrate a phenotypic change, for example decreased production of HBV RNA or protein(s) compared to untreated cells or cells treated with a control siNA. The siNA from cells demonstrating a positive phenotypic change (e.g., decreased HBV RNA or protein), are sequenced to determine the most suitable target site(s) within the target RNA sequence.

Detail Description Paragraph - DETX (122):

Inhibition of HBV Using siNA Molecules of the Invention

Detail Description Paragraph - DETX (127):

[0313] Immulon 4 (Dynax) microtiter wells were coated overnight at 4.degree. C. with anti-HBsAg Mab (Biostride B88-95-31ad,ay) at 1 .mu.g/ml in Carbonate Buffer (Na2CO3 15 mM, NaHCO3 35 mM, pH 9.5). The wells were then washed 4.times. with PBST (PBS, 0.05% Tween.RTM. 20) and blocked for 1 hr at 37.degree. C. with PBST, 1% BSA. Following washing as above, the wells were dried at 37.degree. C. for 30 min. Biotinylated goat ant-HBsAg (Accurate YVS1807) was diluted 1:1000 in PBST and incubated in the wells for 1 hr. at 37.degree. C. The wells were washed 4.times. with PBST.

Streptavidin/Alkaline Phosphatase Conjugate (Pierce 21324) was diluted to 250 ng/ml in PBST, and incubated in the wells for 1 hr. at 37.degree. C. After washing as above, p-nitrophenyl phosphate substrate (Pierce 37620) was added to the wells, which were then incubated for 1 hr. at 37.degree. C. The optical density at 405 nm was then determined. Results of this study are summarized in FIG. 11, where the siNA duplex (sense strand SEQ ID NO: 1338/antisense strand SEQ ID NO: 1342) and inverted control siNA duplex (sense strand SEQ ID NO: 1358/antisense strand SEQ ID NO: 1350) were tested at differing lipid concentrations as indicated in the figure. As shown in FIG. 11, the siRNA construct targeting site 413 of HBV RNA provides significant inhibition of viral replication/activity when compared to an inverted siRNA control. This effect is seen consistently at differing concentrations of lipid transfection agent.

Claims Text - CLTX (3):

2. A short interfering nucleic acid (siNA) molecule that inhibits HBV replication.

US-PAT-NO: 6747050

DOCUMENT-IDENTIFIER: US 6747050 B1

TITLE: Isoxazoline derivative and a process for the preparation thereof

DATE-ISSUED: June 8, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 088288

DATE FILED: March 15, 2002

PARENT-CASE:

This application is a 371 of PCT/KR00/01047 filed Sep. 18, 2000.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
WO	PCT/KR99/00561	September 17, 1999
KR	1999-48608	November 4, 1999

PCT-DATA:
APPL-NO: PCT/KR00/01047
DATE-FILED: September 18, 2000
PUB-NO: WO01/21600
PUB-DATE: Mar 29, 2001
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 514/378, 514/307 , 514/314 , 546/146 , 546/169 , 548/240

ABSTRACT:

The present invention provides to an isoxazoline derivative of formula (I), the pharmaceutically acceptable salts, esters and stereochemically isomeric forms thereof, and the use of the derivative in inhibiting the activity of caspases. The present invention also provides a pharmaceutical composition for preventing inflammation and apoptosis which comprise the isoxazoline derivative, pharmaceutically acceptable salts, esters and stereochemically isomeric forms thereof and the process for preparing the same. The derivative according to the present invention can be effectively used in treating diseases due to caspases, such as, for example the disease in which cells are abnormally

died, dementia, cerebral stroke, AIDS, diabetes, gastric ulcer, hepatic injury by hepatitis, sepsis, organ transplantation rejection reaction and anti-inflammation.

13 Claims, 31 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 18

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Brief Summary Text - BSTX (15):

Several kinds of caspase inhibitors have been identified. Four distinct classes of viral inhibitors have been described: CrmA, p35, a family of IAP (inhibitors of apoptosis), and the hepatitis B virus-encoded HBx protein (See, Gottlob, K et al., 1998, J. Biol. Chem. 273: 33347-33353). However, these molecules are not suitable as the therapeutic agent. Peptide-based caspase inhibitor such as z-VAD-fmk, z-DEVD-fmk, and Ac-YVAD-cmk has widely been used for research use and this inhibitor showed apoptosis-blocking activity in cellular level (See: Sane, A. T. et al., 1998, Cancer Res. 58: 3066-3072), in rodent models of liver injury caused by. Fas or by TNF.alpha. (See: Kunstle, G. et al., 1997, Immunol. Lett. 55; 5-10) or ischemia after liver transplantation (See: Cursio, R. et al., 1999, FASEB J. 13: 253-261). Petak and colleagues showed that a bi-functional anticancer agent, BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea) had caspase inhibiting activity and inhibited drug-induced apoptosis in vitro (See: Petak, I. et al., 1998, Cancer Res. 58: 614-618). Recently, cyclooxygenase-2 (COX-2) inhibitors are arousing interest as potential therapeutic agents of FHF (See, McCormick, P. A. et al., 1999, Lancet 353: 40-41). However, the efficacy of these materials has not been clinically verified yet.

US-PAT-NO: 6709812

DOCUMENT-IDENTIFIER: US 6709812 B1

TITLE: Method for typing and detecting HBV

DATE-ISSUED: March 23, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stuyver; Lieven	Herzele	N/A	N/A	BE
Rossau; Rudi	Ekeran	N/A	N/A	BE
Maertens; Geert	Bruges	N/A	N/A	BE

APPL-NO: 09/ 155885

DATE FILED: October 8, 1998

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	96870053	April 19, 1996

PCT-DATA:
APPL-NO: PCT/EP97/02002
DATE-FILED: April 21, 1997
PUB-NO: WO97/40193
PUB-DATE: Oct 30, 1997
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/5, 435/6 , 536/23.72 , 536/24.32

ABSTRACT:

The present invention relates to a method for detection and/or genetic analysis of HBV in a biological sample, comprising hybridizing the polynucleic acids of the sample with a combination of at least two nucleotide probes, with said combination hybridizing specifically to a mutant target sequence chosen from the HBV RT pol gene region and/or to a mutant target sequence chosen from the HBV preCore region and/or to a mutant target sequence chosen from the HBsAg region of HBV and/or to a HBV genotype-specific target sequence, with said target sequences being chosen from FIG. 1, and with said probes being applied to known locations on a solid support and with said probes being capable of hybridizing to the polynucleic acids of the sample under the same hybridization and wash conditions, or with said probes hybridizing specifically with a sequence complementary to any of said target sequences, or a sequence wherein T of said target sequence is replaced by U; and detecting the hybrids formed; and inferring the HBV genotype and/or mutants present in said sample from the differential hybridization signal(s) obtained. The invention further relates to sets of nucleotide probes and possibly primers useful in said methods as well as to their use in a method for typing and/or detecting HBV and to assay kits using the same.

30 Claims, 28 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 28

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Brief Summary Text - BSTX (6):

Lamivudine is a (-) enantiomer of 3' thiacytidine, a 2'3'-dideoxynucleoside analogue, and is known to be a potent inhibitor of HBV replication through inhibition of the reverse transcriptase (RT) activity of the HBV polymerase. Lamivudine treatment can result in histological improvements in chronic hepatitis patients, and when given pre- and post-liver transplantation, it can prevent graft reinfection (Honkoop et al., 1995; Naoumov et al., 1995). However, after treatment, a hepatitis flare-up can be observed in most patients, with ALT elevations and HBV DNA that becomes detectable again. This HBV DNA rebound is associated with a new quasi species equilibrium. In a few cases, virus breakthrough during therapy was observed, due to the selection of lamivudine resistent HBV strains. The exact nature of this breakthrough has been ascribed to the accumulation of mutations in the RT part of the Polymerase. A similar mechanism in the HIV RT polymerase has been found, where upon lamivudine treatment, mutations accumulate in the YMDD motif (Gao et al., 1993). This YMDD motif is also present in the RT part of the HBV polymerase, and lamivudine-selected mutations in HBV have been found in this region (Tipples et al., 1996), as well as in other regions of the RT part of the polymerase (Ling et al., 1996). Penciclovir is another drug that has been shown to inhibit the reverse transcriptase activity of the HBV polymerase (Shaw et al., 1996), and mutations in the HBV polymerase may also be detected upon treatment with this drug.

Drawing Description Text - DRTX (3):

FIG. 1: Alignment of 35 complete HBV genomes. Isolates belonging to genotype A are: HBVXCPS, HBVADW, HVHEPB, S50225, HPBADWZCG; genotype B: HPBADW3, HPBADWZ, HPBADW1, HPBADW2; genotype C: HPBCGADR, HBVADRM, HPBADRA, HPBCG, HEHBVAYR, HBVADR, HBVADR4, HPBADR1C, HPBADRC, HBVPREX, HPBETNC, HHVBC, HHVCCHA; genotype D: HBVAYWMCG, HBVAYWC, HBVAYWCI, HBVAYWE, HBVDNA, HPBHBVAA, HBVADW4A. To preserve alignment, several gaps were created in the alignment and are indicated with/. Positions of start and end of the different HBV encoded genes is indicated: HBsAg: hepatitis B surface antigen (small surface antigen); HBx: hepatitis B X protein; HB Pol: hepatits B polymerase protein, encoding a terminal protein, a spacer, a RT/DNA polymerase region, and an RNAse H activity; HBcAg: hepatitis B Core antigen; HBpreS1Ag: hepatitis B preS1 antigen (large surface antigen); HBpreS2Ag: hepatitis B preS2 antigen (middle surface antigen). The position of the PCR primers is indicated with a large box over all 35 sequences. The polarity of the PCR primer can be deduced from the position of the name above these boxes: left=antisense primer; right=sense primer. LiPA probes are indicated with small boxes, the numbers of the probes are indicated next to the probes or to the right of the alignment, and correspond to the probe numbers in Table 1.

Drawing Description Paragraph Table - DRTL (1):

TABLE 1 HBV probe and primer design Name Sequence SEQ ID NO Region HBPr1 GGGTCACCATATTCTTGGG 1 preS1 primer sense HBPr2 GAACAAGAGCTACAGCATGGG 2 preS1 primer sense HBPr3 CCACTGCATGGCCTGAGGATG 3 preS1 primer anti-sense HBPr4 GTTCCT/GGAACTGGAGCCACCAG 4 preS1 primer anti-sense HBPr5 TCTTTGTATTAGGAGGCTGTAG 5 preCore primer sense HBPr6 GCTGTAGGCATAAATTGGTCTG 6 preCore primer sense HBPr7 CTCCACAGT/AAGCTCCAAATTC 7 preCore primer anti-sense HBPr8 GAAGGAAAGAAGTCAGAAGGC 8 preCore primer anti-sense HBPr9 TGGCTTTGGGGCATGG 9 preCore HBPr10 TGGCTTTAGGGCATGG 10 preCore HBPr11 TGGCTTTAGGACATGG 11 preCore HBPr12 AAGTTGCATGGTGCTG 12 preCore HBPr13 CACCTCTGCCTAATCAT 13 preCore HBPr14 TGGGGTGGAGCCCTCAG 14 preS1 HBPr15

GCCAGCAGCCAACCAG 15 preS1 HBPr16 CCCATGGGGGACTGT 16 preS1 HBPr17
AACCCCAACAAGGATG 17 preS1 HBPr18 TCCACCAGCAATCCT 18 preS1 HBPr19
TGGGGGAAGAATATTT 19 preS1 HBPr20 AAATTCCAGCAGTCCC 20 preS1 HBPr21
GTTCCCAACCCTCTGG 21 preS1 HBPr22 AACCTCGCAAAGGCAT 22 preS1 HBPr23
TGCATTCAAAGCCAAC 23 preS1 HBPr24 TACTCACAACTGTGCC 24 preS1 HBPr25
ACCCTGCGTTCGGAGC 25 preS1 HBPr26 CAGGAAGACAGCCTAC 26 preS1 HBPr27
GATCCAGCCTTCAGAG 27 preS1 HBPr28 ATGCTCCAGCTCCTAC 28 preS1 HBPr29
GCTTTCTTGGACGGTC 29 preS1 HBPr30 CTACCCCAATCACTCC 30 preS1 HBPr31
AGCACCTCTCTCAACG 31 preS1 HBPr32 CCAATGGCAAACAAGG 32 preS1 HBPr33
CTGAGGGCTCCACCCCA 33 preS1 HBPr34 ATGCAACTTTTTCACC 34 preCore HBPr35
ATCTCTTGTACATGTC 35 preCore HBPr36 ATCTCATGTTCATGTC 36 preCore HBPr37
CAGTGGGACATGTACA 37 preCore HBPr38 CAGTAGGACATGAACA 38 preCore HBPr39
CTGTTCAAGCCTCCAA 39 preCore HBPr40 AGCCTCCAAGCTGTGC 40 preCore HBPr41
AAAGCCACCCAAGGCA 41 preCore HBPr42 TGGCTTTAGGACATGGA 42 preCore HBPr43
GACATGTACAAGAGATGA 43 preCore HBPr44 GACATGAACATGAGATGA 44 preCore HBPr45
TGTACATGTCCCACTGTT 45 preCore HBPr46 TGTTCATGTCCTACTGTT 46 preCore HBPr47
ACTGTTCAAGCCTCCAAG 47 preCore HBPr48 GGCACAGGCTTGGAGGCTT 48 preCore HBPr49
AAAGCCACCCAAGGCACA 49 preCore HBPr50 CCCAGAGGGTTGGGAAC 50 preS1 HBPr51
CAGCATGGGGCAGAATCT 51 preS1 HBPr52 TCCACCAGCAATCCTCTG 52 preS1 HBPr53
GGATCCAGCCTTCAGAGC 53 preS1 HBPr54 TCAGGAAGACAGCCTAC 54 preS1 HBPr55
TTCAACCCCAACAAGGATC 55 preS1 HBPr56 AATGCTCCAGCTCCTAC 56 preS1 HBPr57
CTGCATTCAAAGCCAACT 57 preS1 HBPr58 CCCCATGGGGGACTGTTG 58 preS1 HBPr59
CATACTCACAACTGTGCCA 59 preS1 HBPr60 GGGCTTTCTTGGACGGTCC 60 preS1 HBPr61
CTCTCGAATGGGGGAAGA 61 preS1 HBPr62 CCTACCCCAATCACTCCA 62 preS1 HBPr63
AGCACCTCTCTCAACGACA 63 preS1 HBPr64 GCAAATTCCAGCAGTCCCG 64 preS1 HBPr65
GCCAATGGCAAACAAGGTA 65 preS1 HBPr66 GACATGAACATGAGATG 66 preCore HBPr67
GGACATGAACAAGAGAT 67 preCore HBPr68 GACATGTACAAGAGATG 68 preCore HBPr69
ACATAAGAGGACTCTTGGAC 69 preCore primer sense HBPr70 TACTTCAAAGACTGTGTGTTTA 70
preCore primer sense HBPr71 ACAAAGACCTTTAAC/TCT 71 preCore promoter HBPr72
ACAAAGATCATTAAC/TCT 72 preCore promoter HBPr73 TTCCACCAGCAATCCTC 73 preS1
HBPr74 GATCCAGCCTTCAGAGC 74 preS1 HBPr75 CAAGGTATGTTGCCCGTTTGTCC 75 HBsAg
primer sense HBPr76 CCAAACAGTGGGGGAAAGCCC 76 HBsAg primer anti-sense HBPr77
CTACGGATGGAAATTGC 77 HBsAg codon 145 wild type HBPr78 TACGGACGGAAACTGC 78
HBsAg codon 145 wild type HBPr79 TTCGGACGGAAACTGC 79 HBsAg codon 145 wild
type HBPr80 CTTCGGACGGAAATTGC 80 HBsAg codon 145 wild type HBPr81
CTACGGATAGAAATTGC 81 HBsAg codon 145 mutant HBPr82 CTTCGGACAGAAATTGC 82
HBsAg codon 145 mutant HBPr83 CTATGGGAGTGGGCCTCAGT/CC 83 HB Pol HBPr84
GCTGTAGGCATAAATTGGTCTG 84 preCore primer sense HBPr85 CTCCACAGT/AAGCTCCAAATTC
85 preCore primer anti-sense HBPr86 ACATAAGAGGACTCTTGGAC 86 preCore primer
sense HBPr87 TACTTCAAAGACTGTGTGTTTA 87 preCore primer sense HBPr88
TAGGTTAAAGGTCTTTGT 88 preCore promoter HBPr89 TAGGTTAATGATCTTTGT 89 preCore
promoter HBPr90 CATGTCCCACTGTTCAA 90 preCore HBPr91 CATGTCCTACTGTTCAA 91
preCore HBPr92 TTCTGCCCCATGCTGTA 92 preS1 HBPr93 TTCTGCCCCATGCTGTAG 93
preS1 HBPr94 GGTAA/TAAAGGGACTCAC/AGATG 94 HBsAg primer anti-sense HBPr95
TCAGCTATATGGATGAT 95 HB Pol HBPr96 CAGCTATATGGATGAT 96 HB Pol HBPr97
TTCAGCTATATGGATG 97 HB Pol HBPr98 TCAGTTATATGGATGAT 98 HB Pol HBPr99
TTTCAGTTATATGGATG 99 HB Pol HBPr100 TTTAGTTATATGGATGA 100 HB Pol HBPr101
TCAGCTATGTGGATGAT 101 HB Pol HBPr102 TCAGTTATGTGGATGAT 102 HB Pol HBPr103
TTTCAGCTATGTGGATG 103 HB Pol HBPr104 CAAGGTATGTTGCCCGTTTGTCC 104 HBsAg
primer sense HBPr105 GGT/CAA/TAAAGGGACTCAC/AGATG 105 HBsAg primer anti-sense
HBPr106 GGGTCACCATATTCTTGGG 106 preS1 primer sense HBPr107
GTTCCT/GGAACTGGAGCCACCAG 107 preS1 primer anti-sense HBPr108
CCGGAAAGCTTGAGCTCTTCTTTTTCACCTCTGCCTAATC 108 preCore primer sense HBPr109
CCGGAAAGCTTGAGCTCTTCAAAAAGTTGCATGGTGCTGG 109 preCore primer anti-sense
HBPr110 CCTCTGCCGATCCATACTGCGGAAC 110 preX primer sense HBPr111
CTGCGAGGCGAGGGAGTTCTTCTTC 111 HB Core primer anti-sense HBPr112
TGCCATTTGTTCAGTGGTTCGTAGGGC 112 HBsAg primer sense HBPr113
CCGGCAGATGAGAAGGCACAGACGG 113 HBX primer antisense HBPr114 TTCAGCTATATGGATGAT
114 YMDD motif HBPr115 TCAGCTATATGGATGATG 115 YMDD motif HBPr116

TTCAGCTATGTGGATGAT 116 YMDD motif HBPr117 TCAGCTATGTGGATGATG 117 YMDD motif HBPr118 GGCTTTGGGGCATGG 118 preCore codon 28 wild type HBPr119 TGGCTTTGGGGCATG 119 preCore codon 28 wild type HBPr120 GTGGCTTTGGGGCATG 120 preCore codon 28 wild type HBPr121 GGCTTTGGGGCATGGA 121 preCore codon 28 wild type HBPr122 TGGCTTTGGGACATGG 122 preCore codon 28 wild type, codon 29 mutant HBPr123 GGCTTTGGGACATGG 123 preCore codon 28 wild type, codon 29 mutant HBPr124 TGGCTTTGGGACATG 124

Detailed Description Text - DETX (75):

Shaw, T., Mok, S. S., Locarnini, S. A. Inhibition of hepatitis B virus DNA polymerase by enantiomers of penciclovir triphosphate and metabolic basis for selective inhibition of HBV replication by penciclovir. Hepatology 1996; 24: 996-1002.

US-PAT-NO: 6696423

DOCUMENT-IDENTIFIER: US 6696423 B1

TITLE: Methods and compositions for therapies using genes encoding secreted proteins such as interferon-beta

DATE-ISSUED: February 24, 2004

INVENTOR-INFORMATION:

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APPL-NO: 09/ 512946

DATE FILED: February 25, 2000

PARENT-CASE:

This application is a continuation of PCT application number PCT/US98/17606 filed Aug. 25, 1998 (pending), which claims benefit of U.S. provisional application 60/057,254, filed on Aug. 29, 1997.

US-CL-CURRENT: 514/44, 424/93.1 , 435/320.1 , 435/325 , 435/455 , 536/23.1

ABSTRACT:

Methods and pharmaceutical compositions for modifying cells of a mammalian recipient with DNA encoding a secreted protein such as human interferon in situ are provided. The methods include forming a secreted protein expression system in vivo or ex vivo and administering the expression system to the mammalian recipient. The expression system and methods are useful for the localized and systemic delivery of interferons in situ.

12 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

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Brief Summary Text - BSTX (7):

Interferons also appear to have antiviral activities that are based on two different mechanisms. For instance, type I interferon proteins (.alpha. and .beta.) can directly inhibit the replication of human hepatitis B virus ("HBV") and hepatitis C virus ("HCV"), but can also stimulate an immune response which attacks cells infected with these viruses.

Detailed Description Text - DETX (93):

It is also possible to design a vector in which the interferon gene is only expressed in hepatitis-infected cells. For instance, expression of the interferon gene could be induced by the HBV transcription factor HBx which will only be present in HBV-infected hepatocytes. In addition, a "carrier" system may be used. This could be a non-viral delivery system such as cationic

liposomes or protein:DNA conjugates (conjugates of DNA with asialoglycoproteins can be delivered preferentially to the liver via binding to the asialoglycoprotein receptor on hepatocytes).

US-PAT-NO: 6613883

DOCUMENT-IDENTIFIER: US 6613883 B1

TITLE: Screening assays for compounds that cause apoptosis and related compounds

DATE-ISSUED: September 2, 2003

INVENTOR-INFORMATION:

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APPL-NO: 09/ 248776

DATE FILED: February 12, 1999

PARENT-CASE:

This application is a continuation of Ser. No. 08/675,631, filed Jul. 1, 1996, now U.S. Pat. No. 5,985,829 which is a continuation-in-part of U.S. application Ser. No. 08/359,316, filed on Dec. 19, 1994, now pending.

US-CL-CURRENT: 530/358, 424/185.1 , 424/277.1 , 530/300 , 530/324 , 530/326 , 530/327 , 530/350

ABSTRACT:

This invention relates to methods of screening for compounds capable of inducing apoptosis in certain tumor cells. The invention also relates to compounds identified by such methods. In addition, the invention relates to methods for the in vitro diagnosis of Xeroderma pigmentosum and compounds useful in these methods.

14 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

---------- KWIC ---------

Drawing Description Text - DRTX (5):

FIG. 4. Peptides corresponding to helicase motif III of XPB and the C-terminus of p53 prevent XPB from binding to GST-p53. Four different synthetic peptides were preincubated with 2 .mu.g GST-p53WT for 30 minutes on ice before the addition of .sup.35 S-labeled, in vitro-translated XPB for 60 min at RT. Peptide #464 corresponds to residues 464-478 of XPB (lanes 2-4; 12, 120, and 596 nM), peptide #479 corresponds to residues 479-493 of XPB (lanes 5-7; 12, 116, and 578 nM), peptide #99 corresponds to residues 100-115 of HBX (lanes 8-9; 111 and 554 nM), and peptide #p53cp corresponds to residues 367-387 of p53 (lanes 11-12; 85 and 424 nM).

Detailed Description Text - DETX (63):

In order to demonstrate the binding of several mutant p53 proteins to NER proteins, equal amounts of GST-tagged p53 mutants, (135Y, 249S, 273H) were tested for binding to the in vitro-translated XPD, XPB, CSB and Rad3 proteins used above. The mutant p53 proteins were produced as described in Example 1 and were tested for binding to NER proteins in the same manner as was wild type p53. The results are shown in FIG. 1. All the mutants tested had similar or increased binding to the human proteins but decreased binding to yeast Rad3, as compared to GST-p53-WT (see FIG. 1A, comparing lane 3 with lanes 4, 5, and 6). While not wishing to be bound by theory, this opens the possibility that mutant p53 may exert a dominant negative effect by binding to and sequestering the cellular targets of wild-type p53. The p53-135Y mutant, which has diminished binding to hepatitis B virus X protein and papillomavirus E6 protein, binds to XPD, XPB, Rad3 and CSB. HBX has also been shown to inhibit p53 binding to XPB presumably by binding to the same site of p53 that interacts with XPB, since HBX did not bind to XPB in vitro (see Wang, X. W., et al. (1994) Proc. Natl. Acad. Sci., USA 91:2230-2234.)

Other Reference Publication - OREF (10):
Proc. Natl. Acad. Sci. USA, "Hepatitis B virus X protein inhibits p53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3," Xin Wei Wang, et al., vol. 91, pp. 2230-2234, Mar. 1994 Medical Sciences.

Other Reference Publication - OREF (18):
Wang, X.W., et al. (1994) "Interaction with hepatitis B virus X protein inhibits p53 transcriptional activity and p53 associated with ERCC3", Proceedings of the American Association For Cancer Research, Abstracts 35:585 (3486).

Other Reference Publication - OREF (23):
Wang, X.W., et al. (1994) "Hepatitis B virus X protein inhibits p53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3", Proc. Natl. Acad. Sci. USA, 91:2230-2234.

US-PAT-NO: 6610471

DOCUMENT-IDENTIFIER: US 6610471 B1

TITLE: Methods and compositions to investigate infection by hepatitis B virus and agents to prevent and treat the infection

DATE-ISSUED: August 26, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Isom; Harriet C.	Hershey	PA	N/A	N/A
Delaney, IV; William E.	San Bruno	CA	N/A	N/A

APPL-NO: 09/ 600731

DATE FILED: September 5, 2000

PARENT-CASE:

RELATED APPLICATIONS

This application claims priority to U.S. Ser. No. 60/072017 filed Jan. 21, 1998, said application is incorporated by reference.

PCT-DATA:
APPL-NO: PCT/US99/01153
DATE-FILED: January 20, 1999
PUB-NO: WO99/37821
PUB-DATE: Jul 29, 1999
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/5, 435/370 , 435/69.1 , 436/820

ABSTRACT:

Methods and compositions that use the hepatitis B virus genome, and fragments or extensions, in a baculovirus vector, to develop anti-HBV agents and to drive high-level expression of a desired gene in a cell of hepatic origin.

11 Claims, 50 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 32

---------- KWIC ---------

Brief Summary Text - BSTX (11):

The 2.2.15 cell line which was derived from HepG2 cells and constitutively produces HBV has been used to evaluate in vitro inhibition of HBV replication by various nucleosides. Transient transfection of HepG2 cells have been used to ifs understand various aspects of HBV gene expression and replication at the

molecular level. Some studies involve using greater than genome length HBV DNA sequences so that all HBV gene products can be produced. Transcription of linear HBV DNA requires a greater than unit length 3.2-kb HBV genome to produce the 3.5-kb pregenomic message which is required for replication. Others have concentrated on using HBV DNA sequences which encode restricted portions of the coding regions of the genome or enhancer or promoter sequences.

Detailed Description Text - DETX (10):

Another factor which must be taken into consideration with regard to the level of HBV expression in HBV baculovirus infected HepG2 cells is the content of HBV sequences that are present within the baculovirus. Specifically, the HBV baculovirus was generated using a 1.3-genome length HBV construct, which contains two HBX open reading frames and enhancer promoter regions. It has been previously demonstrated that a 1.2-genome length HBV construct, containing only one HBX open reading frame, is sufficient for generating transgenic mice that express HBV and an HBV-producing cell line. It is possible that the presence of two HBX open reading frames and/or regulatory sequences may have a stimulatory effect on HBV expression by HBV baculovirus.

Detailed Description Text - DETX (20):

The invention includes generation and use of a recombinant baculovirus which includes at least a part of an HBV genome and therefore is capable of initiating HBV replication in the HepG2 cell line. Transcription of linear HBV DNA requires a greater than unit length 3.2-kb HBV genome to produce the 3.5-kb pregenomic message which is required for replication. This has been accomplished by others by developing multimeric or terminally redundant HBV DNA constructs. The recombinant baculovirus of the present invention encodes a 1.3-genome length HBV construct. This construct contains two complete HBV X protein (HBX) open reading frames as well as two enhancer I/X promoter regions and was used because this construct was previously shown to drive high levels of HBV replication in the livers of transgenic mice. (Guidotti et al., 1995) The nature and magnitude of HBV expression and replication obtainable in HepG2 cells infected with the recombinant HBV encoding baculovirus was determined. For comparative purposes, the 2.2.15 cell line was used.

Detailed Description Text - DETX (87):

Treatment of HBV baculovirus infected HepG2 cells with 3TC resulted in an inhibition of HBV replication as evidenced by reductions in the levels of both extracellular HBV DNA and intracellular replicative intermediates. FIG. 19, Table 2 presents an analysis of HBV DNA secreted by HepG2 cells infected with 50 pfu HBV baculovirus/cell and treated with increasing concentrations of 3TC over a 10 day period. Treatments with 0, 0.02, 0.2, and 2.0 .mu.M 3TC were initiated either 16 hours prior to HBV baculovirus infection (P) or 24 hours p.i. (T). After 3 (A), 6 (B), and 9 days (C) of 3TC treatment, DNA was extracted from the medium of each culture and analyzed by Southern Blotting. Relaxed circular (RC) and double-stranded (DS) forms of HBV DNA are indicated. FIG. 20, Table 3 is an analysis of HBV replicative intermediates in HepG2 cells infected with 50 pfu HBV baculovirus/cell and treated with increasing concentrations of 3TC over a 10 day period. Treatments with 0, 0.02, 0.2, and 2.0 .mu.M 3TC were initiated either 16 hours prior to HBV baculovirus infection (P) or 24 hours p.i. (T). After 3 (A), 6 (B), and 9 days (C) of 3TC treatment, replicative intermediates were extracted from cytoplasmic core particles isolated from each culture. Replicative intermediates were analyzed by Southern blotting. Double-stranded (DS) and single-stranded (SS) forms of HBV genomic DNA are indicated. The effect of 3TC was both dose and time dependent and the reductions in extracellular HBV DNA agreed well with the reported efficacy of 3TC in vitro.

Detailed Description Text - DETX (90):

(-)-2'-dideoxy-3'-thiacytidine (3TC, lamivudine) is a nucleoside analog originally described as an agent capable of inhibiting the replication of type 1 and type 2 human immunodeficiency virus (HIV). It was subsequently reported that 3TC was also effective at inhibiting HBV replication in vitro and at reducing the level of serum HBV DNA in vivo in some animal models. 3TC is currently undergoing clinical trials and initial results have been promising. Treatment with 3TC is reported to be well-tolerated and effective at reducing or clearing HBV DNA from the serum of patients. A major drawback to 3TC therapy is that cessation of drug treatment results in the rapid reappearance of serum HBV DNA. The reason for this rebound is postulated to be the persistence of a covalently closed circular (CCC) form of the HBV genome which resides in the nuclei of infected hepatocytes. Although replicative forms of HBV DNA can be prematurely terminated by the incorporation of 3TC, there is little or no evidence to suggest that CCC DNA levels can be affected by treatment with 3TC or other nucleoside analogs. On the contrary, Moraleda et. al. (1997) have reported that established pools of Woodchuck Hepatitis Virus (WHV) CCC DNA produced in vitro in primary woodchuck hepatocytes are quite resistant to 3TC treatment. Recently, 3TC has also been used as a prophylactic agent during orthotopic liver transplant when HBV reinfection is a risk. Short term results from these trials indicate that 3TC suppresses HBV DNA production by the donor liver in some patients; however, the prevention of reinfection and long term efficacy has yet to be determined. It is unknown if preemptive treatment with nucleosides such as 3TC prior to transplant would be sufficient to prevent the accumulation of CCC HBV DNA in new liver tissue and potential reactivation of the virus.

Detailed Description Text - DETX (91):

The HBV baculovirus-HepG2 cell system also allows the detection of CCC HBV DNA which can be difficult to detect or undetectable in stably transfected HBV expressing cell lines such as HepG2 2.2.15 and HB611. Unlike stable cell lines, the time of infection can also be controlled allowing the manipulation or treatment of cells prior to or during the initiation of HBV expression. The primary goals of the studies described here were: (1) to evaluate the utility of the HBV baculovirus-HepG2 system as a tool for antiviral research using 3TC, an established inhibitor of HBV replication; (2) to provide further data on the in vitro efficacy of 3TC by investigating its effects on various levels of HBV replication; and (3) to examine the effect of administering 3TC prior to the initiation of HBV expression on viral replication and the accumulation of CCC HBV DNA.

Detailed Description Text - DETX (93):

When testing viral mRNA synthesis and HBV antigen secretion by HBV baculovirus infected HepG2 cells, no differences were found between treated and untreated cultures. (FIGS. 21 and 22) This has also been reported by other investigators and is not unexpected based on the mechanism by which 3TC acts. 3TC is phosphorylated inside cells and is subsequently incorporated into nascent viral DNA by the HBV polymerase during replication. 3TC incorporation results in the termination of DNA elongation by virtue of its dideoxy structure. Therefore, the respected result would be that 3TC would not directly affect the transcription or translation of HBV gene products from nuclear DNA because it acts downstream of these events. It is interesting that an almost complete inhibition of the presence of extracellular HBV DNA did not result in any discernible alteration in the trafficking or secretion of HBeAg or HBsAg in HepG2 cells. This effect is also observed in patients, the majority of whom do not clear either HBeAg or HBsAg after long term treatment with 3TC even though their serum HBV DNA levels are markedly reduced.

Detailed Description Text - DETX (98):

Unlike replicative intermediates and extracellular HBV DNA which were

progressively inhibited with increasing time of 3TC treatment, RC and CCC DNA did not undergo a consistent decrease relative to control levels as the length of time of 3TC treatment increased. This finding suggests that 1) CCC HBV DNA, after formation, is fairly stable within the nuclei of infected HepG2 cells, and/or 2) Mature core particles can cycle back to the nucleus to offset CCC DNA turnover and maintain a small pool of episomal HBV genomes, even when HBV DNA replication was drastically suppressed as evidenced by the levels of extracellular HBV DNA and HBV replicative intermediates. If the later hypothesis is correct, it is possible that when very few mature HBV cores are present in the cytoplasm, there is a tendency for those cores to enter the CCC amplification pathway instead of acquiring an envelope and exiting the cell. Indeed, the finding that extracellular HBV DNA levels are suppressed to a greater extent than intracellular replicative intermediates provides support for this hypothesis. This would not be unlike the early stages of hepadnaviral replication when the initial cores produced after infection are believed to cycle back to the nucleus to allow an amplification of CCC DNA before the secretion of virions takes place.

Detailed Description Text - DETX (100):

One limitation of using stable cell lines, such as HepG2 2.2.15 cells, for evaluating the efficacy of an antiviral on HBV replication is that the magnitude of virus replication is at a static level predetermined by the number of integrated HBV genome copies. This limitation does not exist when HBV replication in HepG2 cells is mediated by recombinant HBV baculovirus, because it is possible to modulate the level of production of HBV virions over several magnitudes simply by altering the baculovirus multiplicity of infection. In this study, the effects of 3TC on HBV replication were studied using input multiplicities of recombinant HBV baculovirus that varied over a 16 fold range. (FIG. 24, Table 5) FIG. 24 shows the effect of 0.2 .mu.M 3TC on the HBV DNA content of medium from HepG2 cells expressing increasing levels of HBV. HepG2 cultures were infected with 25, 50, 100, 200, and 400 pfu HBV baculovirus/cell and were treated with 0.2 .mu.M 3TC starting 24 hours p.i. Cultures were fed fresh 3TC supplemented medium daily. After 3 days of treatment (4 days p.i.). DNA was extracted from the medium of treated and untreated control cultures and analyzed by Southern Blotting. Autoradiograms shown indicate different elngths of time of exposure of the blot to film: 1 hour (A), 2 hours (B), 4 hours (C), and 8 hours (D). Relaxed circular (RC) and double-stranded (DS) forms of HBV DNA are indicated. The largest reduction of extracellular HBV DNA (>99% reduction after six days of treatment) occurred in cells infected with 25 pfu of baculovirus, the lowest multiplicity tested. Cultures infected at multiplicities ranging from 50 pfu/cell to as high as 400 pfu/cell showed an average reduction of extracellular HBV DNA of greater than 96% after six days of 3TC treatment. This finding was unexpected and clearly indicated that 0.2 .mu.M 3TC was highly effective at inhibiting HBV replication even in the presence of large amounts of the virus. However, it is also important to note that even a 96% reduction in HBV replication still allowed high levels of HBV virions to be secreted from 3TC-treated cells which were replicating very high levels of HBV (i.e. cells infected with HBV baculovirus at a high moi).

Detailed Description Text - DETX (131):

DOCUMENTS CITED Boyce F M, Bucher N L. Baculovirus-mediated gene transfer into mammalian cells. Proc Nat Acad Sci 1996; 93: 2348-2352. Doong S L, Tsai C H, Schinazi R F, Liotta D C, Cheng Y C. Inhibition of the replication of hepatitis B virus in vitro by 2',3'-dideoxy-3'-thiacytidine and related analogues. Proc Nat Acad Sci 1991; 88:8495-8499. Guidotti L G, Matzke B, Schaller H, Chisari F V. High-level hepatitis B virus replication in transgenic mice. J Virol 1995; 69: 6158-6169. Hofmann C, Sandig V, Jennings G, Rudolph M, Schiag P, Strauss M. Efficient gene transfer into human hepatocytes by baculovirus vectors. Proc Nat Acad Sci 1995; 92:10099-10103. Knowles B B,

Howe C C, Aden D P. Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen. Science 1980; 209:497-499. Korba B E, Gerin J L. Use of a standardized cell culture assay to assess activities of nucleoside analogs against hepatitis B virus replication. Antiviral Res 1992; 19:55-70. Kruining J, Heijtink R A, Schalm S W. Antiviral agents in hepatitis B virus transfected cell lines: inhibitory and cytotoxic effect related to time of treatment. Journal of Hepatology 1995; 22:263-267. Moraleda G, Saputelli J, Aldrich C E, Averett D, Condreay L, Mason W S. Lack of effect of antiviral therapy in nondividing hepatocyte cultures on the closed circular DNA of woodchuck hepatitis virus. J Virol 1997; 71:9392-35 9399. Nakabayashi H, Taketa K, Miyano K, Yamane T, Sato J. Growth of human hepatoma cells lines with differentiated functions in chemically defined medium. Cancer Research 1982; 42:3858-3863. Sandig V, Hofmann C, Steinert S, Jennings G, Schlag P, Strauss M. Gene transfer into hepatocytes and human liver tissue by baculovirus vectors. Hum Gene Ther 1996; 7: 1937-1945. Sells M A, Chen M L, Acs G. Production of hepatitis B virus particles in Hep G2 cells transfected with cloned hepatitis B virus DNA. Proc Nat Acad Sci 1987; 84:1005-1009. Sells M A, Zelent A Z, Shvartsman M, Acs G. Replicative intermediates of 50 hepatitis B virus in HepG2 cells that produce infectious virions. J Virol 1988; 62:2836-2844.

US-PAT-NO: 6602979

DOCUMENT-IDENTIFIER: US 6602979 B1

TITLE: Screening assays for compounds that cause apoptosis

DATE-ISSUED: August 5, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Harris; Curtis C.	Bethesda	MD	N/A	N/A
Wang; Xin Wei	North Potomac	MD	N/A	N/A
Hoeijmakers; Jan H. J.	Zevenhuizen	N/A	N/A	NL

APPL-NO: 08/ 359316

DATE FILED: December 19, 1994

US-CL-CURRENT: 530/326, 530/350

ABSTRACT:

This invention relates to methods of screening for compounds capable of inducing apoptosis in certain tumor cells. The invention also relates to compounds identified by such methods. In addition, the invention relates to methods for the in vitro diagnosis of Xeroderma pigmentosum and compounds useful in these methods.

2 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

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Drawing Description Text - DRTX (5):

FIG. 4. Peptides corresponding to helicase motif III of XPB and the C-terminus of p53 prevent XPB from binding to GST-p53. Four different synthetic peptides were pre-incubated with 2 .mu.g GST-p53WT for 30 minutes on ice before the addition of .sup.35 S-labeled, in vitro-translated XPB for 60 min at RT. Peptide #464 corresponds to residues 464-478 of XPB (lanes 2-4; 12, 120, and 596 nM), peptide #479 corresponds to residues 479-493 of XPB (lanes 5-7; 12, 116, and 578 nM), peptide #99 corresponds to residues 100-115 of HBX (lanes 8-9; 111 and 554 nM), and peptide #p53cp corresponds to residues 367-387 of p53 (lanes 11-12; 85 and 424 nM).

Detailed Description Text - DETX (52):

In order to demonstrate the binding of several mutant p53 proteins to NER proteins, equal amounts of GST-tagged p53 mutants, (135Y, 249S, 273H) were tested for binding to the in vitro-translated XPD, XPB, CSB and Rad3 proteins used above. The mutant p53 proteins were produced as described in Example 1 and were tested for binding to NER proteins in the same manner as was wild type p53. The results are shown in FIG. 1. All the mutants tested had similar or increased binding to the human proteins but decreased binding to yeast Rad3, as compared to GST-p53-WT (see FIG. 1A, comparing lane 3 with lanes 4, 5, and 6).

While not wishing to be bound by theory, this opens the possibility that mutant p53 may exert a dominant negative effect by binding to and sequestering the cellular targets of wild-type p53. The p53-135Y mutant, which has diminished binding to hepatitis B virus X protein and papillomavirus E6 protein, binds to XPD, XPB, Rad3 and CSB. HBX has also been shown to inhibit p53 binding to XPB presumably by binding to the same site of p53 that interacts with XPB, since HBX did not bind to XPB in vitro (see Wang, X. W., et al. (1994) Proc. Natl. Acad. Sci., USA 91:2230-2234.)

Other Reference Publication - OREF (6):

Proc. Natl. Acad. Sci. USA, "Hepatitis B virus X protein inhibits p53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3," Xin Wei Wang, et al., vol. 91, pp. 2230-2234, Mar. 1994 Medical Sciences.

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	174	hbx	US-PGPUB; USPAT	OR	OFF	2004/09/21 09:05
L2	492	(hepatitis adj b adj virus or hbv) near4 inhibit$8	US-PGPUB; USPAT	OR	OFF	2004/09/21 09:07
L3	26	1 and 2	US-PGPUB; USPAT	OR	OFF	2004/09/21 09:07
L4	11	hbx near4 inhibit$8	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:02

PGPUB-DOCUMENT-NUMBER: 20040138119

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040138119 A1

TITLE: Use of hepatitis B X-interacting protein (HBXIP) in modulation of apoptosis

PUBLICATION-DATE: July 15, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Tamm, Ingo	Berlin	CA	DE	
Reed, John C.	Rancho Santa Fe		US	

APPL-NO: 10/ 665975

DATE FILED: September 18, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60412109 20020918 US

US-CL-CURRENT: 514/12

ABSTRACT:

Novel methods of regulating cellular apoptosis by affecting the interaction of hepatitis B X-interacting protein (HBXIP) with Survivin are described. More specifically, these novel methods of enhancing apoptosis of neoplastic cells comprises inhibiting interaction of hepatitis B X-interacting protein (HBXIP) with Survivin.

RELATED APPLICATIONS

[0001] This application claims the benefit of priority under 35 U.S.C. 119(e) of the U.S. Provisional Application 60/412,109 filed Sep. 18, 2002, the disclosure of which is expressly incorporated herein by reference in its entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX (197):

[0187] To further explore whether HBX suppresses Caspase activity through a Survivin-dependent mechanism, endogenous Survivin was immunodepleted from HepG2 cell lysates using anti-Survivin antisera or preimmune serum (CTR) and then equivalent amounts analyzed by immunoblotting using anti-Survivin antibody (top panel). Further, equivalent volumes of extracts were analyzed for Caspase activity based on Ac-DEVD-AFC cleavage, where lysates were incubated with recombinant HBX (+) or control protein (-) prior to stimulation with Cytochrome c and dATP. In extracts subjected to mock immunodepletion, HBX suppressed Caspase activation by roughly half (FIG. 6f). In contrast, when Survivin was immunodepleted, it was found that Cytochrome C was more potent at activating Caspases and that HBX had little inhibitory activity (FIG. 6f). Thus, HBX fails to inhibit Caspases in the absence of Survivin.

Claims Text - CLTX (17):

16. A method for identifying an agent that effectively inhibits the activity of HBX, comprising: contacting HBX with a cell extract containing Survivin and pro-Caspase-9 in the presence of a compound; inducing activation of pro-Caspase-9; and measuring the activation of pro-Caspase-9, thereby determining whether said compound is an agent that effectively inhibits the activity of HBX.

PGPUB-DOCUMENT-NUMBER: 20040115747

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040115747 A1

TITLE: Screening assays for compounds that cause apoptosis

PUBLICATION-DATE: June 17, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Harris, Curtis C.	Bethesda	MD	US	
Wang, Xin Wei	North Potomac	MD	US	
Hoeijmakers, Jan H.J.	Zevenhuizen		NL	

APPL-NO: 10/ 633789

DATE FILED: August 4, 2003

RELATED-US-APPL-DATA:

child 10633789 A1 20030804

parent division-of 08359316 19941219 US GRANTED

parent-patent 6602979 US

US-CL-CURRENT: 435/7.23

ABSTRACT:

This invention relates to methods of screening for compounds capable of inducing apoptosis in certain tumor cells. The invention also relates to compounds identified by such methods. In addition, the invention relates to methods for the in vitro diagnosis of Xeroderma pigmentosum and compounds useful in these methods.

---------- KWIC ---------

Detail Description Paragraph - DETX (50):

[0069] In order to demonstrate the binding of several mutant p53 proteins to NER proteins, equal amounts of GST-tagged p53 mutants, (135Y, 249S, 273H) were tested for binding to the in vitro-translated XPD, XPB, CSB and Rad3 proteins used above. The mutant p53 proteins were produced as described in Example 1 and were tested for binding to NER proteins in the same manner as was wild type p53. The results are shown in FIG. 1. All the mutants tested had similar or increased binding to the human proteins but decreased binding to yeast Rad3, as compared to GST-p53-WT (see FIG. 1A, comparing lane 3 with lanes 4, 5, and 6). While not wishing to be bound by theory, this opens the possibility that mutant p53 may exert a dominant negative effect by binding to and sequestering the cellular targets of wild-type p53. The p53-135Y mutant, which has diminished binding to hepatitis B virus X protein and papillomavirus E6 protein, binds to XPD, XPB, Rad3 and CSB. HBX has also been shown to inhibit p53 binding to XPB presumably by binding to the same site of p53 that interacts with XPB, since HBX did not bind to XPB in vitro (see Wang, X. W., et al. (1994) Proc. Natl. Acad. Sci., USA 91:2230-2234.)

PGPUB-DOCUMENT-NUMBER: 20040028684

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040028684 A1

TITLE: Cancer diagnosis and assays for screening anti-cancer agents

PUBLICATION-DATE: February 12, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Gstaiger, Matthias Georg	Basel		CH	
Christian	Riehen		CH	
Krek, Wilhelm				

APPL-NO: 10/ 276016

DATE FILED: November 12, 2002

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
GB	0011439.7	2000GB-0011439.7	May 12, 2000

PCT-DATA:
APPL-NO: PCT/EP01/05411
DATE-FILED: May 11, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 424/155.1, 530/350 , 530/388.8

ABSTRACT:

A novel RMP homologue is provided, as well as nucleic acids encoding the protein and antisense nucleic acids, Methods of diagnosing conditions dependent on RMP are provided as well as screening methods for identify agents active against conditions dependent on RMP, such as conditions dependent on amino acid regulation, cancer, neurodegeneration, muscle degeneration, immune disorders, and AIDS.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (17):
[0017] It is thought that RNA polymerase subunits might provide targets for transcriptional regulators in controlling gene expression. The RNA polymerase II subunit 5 (RPB5) has been demonstrated to interact with RPB5-mediating protein (RMP), a protein that inhibits the trans-activation function of Hbx when overexpressed (Dorjsuren D. et al (1998) Molecular Cell Biol. 18: 7546-7555).

US-PAT-NO: 6613883

DOCUMENT-IDENTIFIER: US 6613883 B1

TITLE: Screening assays for compounds that cause apoptosis and related compounds

DATE-ISSUED: September 2, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Harris; Curtis C.	Bethesda	MD	N/A	N/A
Wang; Xin Wei	North Potomac	MD	N/A	N/A
Hoeijmakers; Jan H. J.	Zevenhuizen	N/A	N/A	NL

APPL-NO: 09/ 248776

DATE FILED: February 12, 1999

PARENT-CASE:

This application is a continuation of Ser. No. 08/675,631, filed Jul. 1, 1996, now U.S. Pat. No. 5,985,829 which is a continuation-in-part of U.S. application Ser. No. 08/359,316, filed on Dec. 19, 1994, now pending.

US-CL-CURRENT: 530/358, 424/185.1 , 424/277.1 , 530/300 , 530/324 , 530/326 , 530/327 , 530/350

ABSTRACT:

This invention relates to methods of screening for compounds capable of inducing apoptosis in certain tumor cells. The invention also relates to compounds identified by such methods. In addition, the invention relates to methods for the in vitro diagnosis of Xeroderma pigmentosum and compounds useful in these methods.

14 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

---------- KWIC ---------

Detailed Description Text - DETX (63):

In order to demonstrate the binding of several mutant p53 proteins to NER proteins, equal amounts of GST-tagged p53 mutants, (135Y, 249S, 273H) were tested for binding to the in vitro-translated XPD, XPB, CSB and Rad3 proteins used above. The mutant p53 proteins were produced as described in Example 1 and were tested for binding to NER proteins in the same manner as was wild type p53. The results are shown in FIG. 1. All the mutants tested had similar or increased binding to the human proteins but decreased binding to yeast Rad3, as compared to GST-p53-WT (see FIG. 1A, comparing lane 3 with lanes 4, 5, and 6). While not wishing to be bound by theory, this opens the possibility that mutant p53 may exert a dominant negative effect by binding to and sequestering the cellular targets of wild-type p53. The p53-135Y mutant, which has diminished

binding to hepatitis B virus X protein and papillomavirus E6 protein, binds to XPD, XPB, Rad3 and CSB. HBX has also been shown to inhibit p53 binding to XPB presumably by binding to the same site of p53 that interacts with XPB, since HBX did not bind to XPB in vitro (see Wang, X. W., et al. (1994) Proc. Natl. Acad. Sci., USA 91:2230-2234.)

US-PAT-NO: 6602979

DOCUMENT-IDENTIFIER: US 6602979 B1

TITLE: Screening assays for compounds that cause apoptosis

DATE-ISSUED: August 5, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Harris; Curtis C.	Bethesda	MD	N/A	N/A
Wang; Xin Wei	North Potomac	MD	N/A	N/A
Hoeijmakers; Jan H. J.	Zevenhuizen	N/A	N/A	NL

APPL-NO: 08/ 359316

DATE FILED: December 19, 1994

US-CL-CURRENT: 530/326, 530/350

ABSTRACT:

This invention relates to methods of screening for compounds capable of inducing apoptosis in certain tumor cells. The invention also relates to compounds identified by such methods. In addition, the invention relates to methods for the in vitro diagnosis of Xeroderma pigmentosum and compounds useful in these methods.

2 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

---------- KWIC ---------

Detailed Description Text - DETX (52):

In order to demonstrate the binding of several mutant p53 proteins to NER proteins, equal amounts of GST-tagged p53 mutants, (135Y, 249S, 273H) were tested for binding to the in vitro-translated XPD, XPB, CSB and Rad3 proteins used above. The mutant p53 proteins were produced as described in Example 1 and were tested for binding to NER proteins in the same manner as was wild type p53. The results are shown in FIG. 1. All the mutants tested had similar or increased binding to the human proteins but decreased binding to yeast Rad3, as compared to GST-p53-WT (see FIG. 1A, comparing lane 3 with lanes 4, 5, and 6). While not wishing to be bound by theory, this opens the possibility that mutant p53 may exert a dominant negative effect by binding to and sequestering the cellular targets of wild-type p53. The p53-135Y mutant, which has diminished binding to hepatitis B virus X protein and papillomavirus E6 protein, binds to XPD, XPB, Rad3 and CSB. HBX has also been shown to inhibit p53 binding to XPB presumably by binding to the same site of p53 that interacts with XPB, since HBX did not bind to XPB in vitro (see Wang, X. W., et al. (1994) Proc. Natl. Acad. Sci., USA 91:2230-2234.)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	174	hbx	US-PGPUB; USPAT	OR	OFF	2004/09/21 09:05
L2	492	(hepatitis adj b adj virus or hbv) near4 inhibit$8	US-PGPUB; USPAT	OR	OFF	2004/09/21 09:07
L3	26	1 and 2	US-PGPUB; USPAT	OR	OFF	2004/09/21 09:07
L4	11	hbx near4 inhibit$8	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:02
L5	10160	src	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:08
L6	794441	activat$8	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:08
L7	239593	upstream	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:09
L8	3912	6 near5 7	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:09
L9	27	8 same 5	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:09

PGPUB-DOCUMENT-NUMBER: 20040146903

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040146903 A1

TITLE: Methods and compositions for modulating activator protein 1

PUBLICATION-DATE: July 29, 2004

INVENTOR-INFORMATION:

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Hogenesch, John B.	Encinitas	CA	US	
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APPL-NO: 10/ 702112

DATE FILED: November 5, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60471864 20030520 US

non-provisional-of-provisional 60425344 20021107 US

US-CL-CURRENT: 435/6, 435/7.2 , 514/44

ABSTRACT:

This invention provides novel AP-1 modulatory polypeptides. The invention also provides methods for screening modulators of AP-1 transcription factor activities. The methods comprise first screening test agents for modulators of an AP-1-modulatory polypeptide and then further screening the identified modulating agents for modulators of AP-1 transcription factor activities. The invention further provides methods and pharmaceutical compositions for modulating AP-1 transcription factor activities in a cell and for treating diseases and conditions mediated by abnormal cellular proliferation.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Patent Application Serial Nos. 60/425,344 (filed Nov. 7, 2002) and 60/471,864 (filed May 20, 2003). The full disclosures of these applications are incorporated herein by reference in their entirety and for all purposes.

---------- KWIC ---------

Detail Description Paragraph - DETX (150):

[0163] To refine the mode of action by which the novel growth regulatory proteins are modulating the AP-1 pathway, their relative inductive activities were examined in the presence of small inhibitor RNA (siRNA) molecules directed against known components of AP-1 signaling. AP-1(PMA) reporter gene activities of pairwise cDNA (novel activators) and siRNA (AP-1 pathway members) transfections were normalized to appropriate controls and analyzed through

single-linkage hierarchical clustering (FIG. 2A). For comparison, effects of the inhibitory RNAs upon PMA-induced AP-1 activity are also shown. siRNAs encoding PI-3 kinase (PIK3R3, PIK3CD), PKC (PKCD, PRKACA, DGKI, MEKK2), and RAS (SRC, RAF) pathway members were most proficient in abrogating the AP-1 induction by the bulk of the identified growth regulators. This demarcates a majority of identified AP-1 activators as functioning upstream of these proteins, and suggests that they regulate AP-1 activity through modulation of PI-3 Kinase, PKC, and/or RAS pathways. For example, the results indicate that the activity of BLK upon the AP-1 reporter gene was extinguished by siRNAs coding for ERK pathway effectors DGKI, MEKK1, SRC, RAF1 and PIK3R3, but not by siRNAs directed against JNK-related pathway molecules MAP3K7, MAPK9, and MAP4K4. These observed activities are consistent with reports that these and related family members are important for AP-1 response to environmental growth stimuli.

PGPUB-DOCUMENT-NUMBER: 20040106156

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040106156 A1

TITLE: Methods and compositions for detecting receptor-ligand interactions in single cells

PUBLICATION-DATE: June 3, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 346620

DATE FILED: January 16, 2003

RELATED-US-APPL-DATA:

child 10346620 A1 20030116

parent continuation-in-part-of 10193462 20020710 US PENDING

non-provisional-of-provisional 60304434 20010710 US

non-provisional-of-provisional 60310141 20010802 US

US-CL-CURRENT: 435/7.2

ABSTRACT:

The invention provides methods and compositions for simultaneously detecting the activation state of a plurality of proteins in single cells using flow cytometry. The invention further provides methods and compositions of screening for bioactive agents capable of coordinately modulating the activity of a plurality of proteins in single cells. The methods and compositions can be used to determine the protein activation profile of a cell for predicting or diagnosing a disease state, and for monitoring treatment of a disease state.

[0001] This application is a continuation in part of U.S. Ser. No. 10/193,462, filed Jul. 10, 2002, which claims the benefit of the filing date of U.S. Ser. No. 60/304,434, filed Jul. 10, 2001, and U.S. Ser. No. 60/310,141, filed Aug. 2, 2001.

---------- KWIC ---------

Detail Description Paragraph - DETX (206):

[0233] The present inventors undertook flow cytometric based p44/42 MAPK kinase inhibition and activation profiling to identify necessary components for LFA-1 signaling. PKC inhibitor BIM I, cytoskeletal disrupting agents cytochalisin D, taxol, nocodozole, and sequestering of divalent cations by EDTA diminished the ICAM-2 induced p44/42 MAPK signal (FIG. 3D), suggesting that the

ligand-induced events of LFA-1 are mechanically linked to signal transduction by the actin-microtubule cytoskeleton. To identify upstream kinases that were responsible for signal transmission from LFA-1 to p44/42 MAPK, a series of kinase inhibitors were applied and tested for their ability to abrogate the ICAM-2 induced p44/42 MAPK activity (FIGS. 2H-I), whereas Herbimycin A and Emodin, inhibitors of src and p56lck had no effect. Tyrphostin A9 and piceatannol, specific inhibitors of proline-tyrosine kinase 2 (Pyk2) and Spleen-tyrosine kinase (Syk), respectively (Avdi, et al. (2001) J Biol Chem 276, 2189-2199.; Fuortes, et al., (1999) J Clin Invest 104, 327-335) abrogated the ICAM-2 induced activation of p44/42 MAPK and its upstream activator Raf-1 (FIG. 4A).

PGPUB-DOCUMENT-NUMBER: 20040072228

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040072228 A1

TITLE: Card11 NFkB activating polypeptides, nucleic acids, inbred and transgenic animals, and methods of use thereof

PUBLICATION-DATE: April 15, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 632696

DATE FILED: August 1, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60401078 20020802 US

non-provisional-of-provisional 60422614 20021029 US

US-CL-CURRENT: 435/6, 435/194 , 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

The invention provides novel caspase recruitment domain 11 (CARD11), also known as CARMA-1, polypeptides, nucleic acids encoding them and methods for making and using them. In one aspect, the polypeptides of the invention have NFkB activating activity. The invention also provides non-human transgenic animals, e.g., mice, comprising the CARD11 nucleic acids of the invention. The invention also provides pharmaceutical compositions comprising a nucleic acid or polypeptide of the invention. Administration of a pharmaceutical composition of the invention to a subject is used to generate a toleragenic immunological environment in the subject. This can be used to tolerize the subject to an antigen. The invention also provides inbred mouse strains homozygous for a non-wild type CARD11 allele. This genotype results in mice having a phenotype comprising dermatitis, B cell defects and T cell defects.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (4):

[0002] The caspase recruitment domain (CARD) polypeptide can function as a protein-binding module to mediate the assembly of CARD-containing proteins into apoptosis and NF-kappaB signaling complexes. It has been reported that CARD protein 11 (CARD11), also known as CARMA-1, and CARD protein 14 (CARD14) maybe members of a membrane-associated guanylate kinase (MAGUK) family, a class of proteins that functions as molecular scaffolds for the assembly of multiprotein complexes at specialized regions of the plasma membrane. CARD11 and CARD14 have been reported to have homologous structures consisting of an N-terminal CARD domain, a central coiled-coil domain, and a C-terminal tripartite domain

comprised of a PDZ domain, an Src homology 3 domain, and a GUK domain with homology to guanylate kinase. The CARD domains of both CARD11 and CARD14 may associate with the CARD domain of BCL10, a signaling protein that activates NF-kappaB through the I-kappa-B kinase complex in response to upstream stimuli. It was reported that when expressed in cells, CARD11 and CARD14 activate NF-kappa B and induce the phosphorylation of BCL11. These findings suggest that CARD11 and CARD14 are MAGUK family members that function as upstream activators of BCL10 and NF-kappaB signaling. See, e.g., Bertin (2001) J. Biol. Chem. 276(15):11877-11882; WO 01/40468.

PGPUB-DOCUMENT-NUMBER: 20040028661

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040028661 A1

TITLE: Expansion of cells using thrombopoietin and anti-transforming growth factor-beta

PUBLICATION-DATE: February 12, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bartelmez, Stephen H.	Seattle	WA	US	

APPL-NO: 10/ 213957

DATE FILED: August 7, 2002

US-CL-CURRENT: 424/93.21, 435/366 , 514/44

ABSTRACT:

The invention features a method for the expansion of hematopoietic stem cells using a combination of a thrombopoietin agonist and a transforming growth factor-beta blocking agent in the absence of stem cell factor. The invention also features a hematopoictic stem cell composition that has been expanded using a combination of a thrombopoietin agonist and a transforming growth factor-beta blocking agent in the absence of stem cell factor, as well as methods of using an expanded hematopoietic stem cell composition to restore or supplement an immune system and/or blood forming system compromised by, for example, radiation or chemotherapy.

---------- KWIC ---------

Detail Description Paragraph - DETX (21):

[0053] By way of example, TPO agonists that activate the mpl receptor signal transduction pathway would activate a JAK, for example, JAK1, JAK2, JAK3 and TYK2, which are activated upon ligand binding to the mpl receptor. Activation of JAKs results in phosphorylation of multiple cellular proteins, including the associated cytokine receptors and the JAKs themselves (reviewed in Ihle, Adv. Immunol., 60:1-35, 1995). The phosphorylated tyrosines are potential docking sites for proteins containing specific phosphotyrosine binding domains (e.g. Src homology (SH)2 and phosphotyrosine binding (PTB) domains). Specific signaling proteins are thereby recruited into the cytokine signaling networks. Since JAK2 physically associates with its activating receptors (via the proline-rich region) and is activated within seconds after receptor engagement, it appears that JAK2 activation is an early, perhaps initiating step in signal transduction by mpl receptor-ligand interaction. A number of signaling molecules that appear to be activated by recruitment to JAK2-mpl-receptor complexes include: 1) Shc proteins, which lie upstream of Ras and the mitogen-activated protein (MAP) kinases ERKs 1 and 2, which are implicated in the regulation of cellular growth and/or differentiation. 2) the insulin receptor substrates (IRS) 1 and 2; and 3) the signal transducers and activators of transcription (STAT) 1, 3, 5a, and 5b, which have been implicated as regulators of transcription of a variety of genes. Agonists of the mpl receptor signaling pathway that are able to activate the JAK/STAT pathway at

the level of protein-protein interaction or protein-DNA interaction are also included in the methods of the invention.

PGPUB-DOCUMENT-NUMBER: 20030224398

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030224398 A1

TITLE: Purification of the leading front of migratory cells

PUBLICATION-DATE: December 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Klemke, Richard L.	San Diego	CA	US	

APPL-NO: 10/ 365961

DATE FILED: February 12, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60356893 20020213 US

US-CL-CURRENT: 435/6, 435/317.1 , 435/325 , 435/4 , 435/7.2 , 514/54

ABSTRACT:

The invention relates generally to methods of modulating cell migration. Included in the methods of the invention are methods of identifying the state of a pseudopodium in cell migration and methods of inducing extension or retraction of a pseudopodia from a cell. The invention also relates to methods of screening for and identifying an agent effective in inducing extension or retraction of a pseudopodium and therefore affecting cell migration. Agents that can modulate cell migration are useful in treatment of conditions in which cell migration plays a role. Such conditions can include wound healing, angiogenesis, and metastasis of a disease from one location to another. Additionally, the invention provides methods of biochemically separating the pseudopodium of a cell from the remainder of the cell body and methods of determining the proteins present in the pseudopodium and cell body. The invention also includes a pseudopodium isolated by the methods of the invention.

RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. .sctn. 119(e) to U.S. Ser. No. 60/356,893, filed Feb. 13, 2002, the entire content of which is incorporated herein by reference.

---------- KWIC ---------

Detail Description Paragraph - DETX (40):

[0064] By the methods of the invention, it was demonstrated that CAS and Crk are specifically assembled during pseudopodia growth and then disassembled during retraction. This process occurred without apparent change in the overall level of CAS tyrosine phosphorylation or FAK activation, which is an upstream activator of CAS (Vuori et al., 1996; Tachibana et al., 1997). One explanation is that Crk couples only to a specific subset of phosphotyrosine residues present in the substrate domain of CAS, which changes during

pseudopodia growth and retraction. CAS/Crk association is mediated through the binding of the SH2 domain of Crk to phosphotyrosine residues present in the substrate domain of CAS (Matsuda et al., 1993). In fact, there are 15 tyrosine residues in this region of CAS that correspond to potential SH2 binding motifs, 9 of which conform to the Crk SH2 recognition sequence YD(V/T)P (Klemke et al., 1998). Alternatively, regulation may occur through serine phosphorylation of CAS (Ma et al., 2001) or phosphorylation of the regulatory tyrosine 221 of Crk, which prevents CAS/Crk coupling in cells (Kain and Klemke, 2001). However, the latter is unlikely as no significant change in Crk tyrosine phosphorylation was detected. The upstream and downstream components that modulate the assembly/disassembly of this molecular scaffold in the pseudopodium are not yet clear, but likely candidates include c-src, PTP-PEST, and PTP-1B (Garton et al., 1996; Liu et al., 1996; Vuori et al., 1996).

PGPUB-DOCUMENT-NUMBER: 20030224360

PGPUB-FILING-TYPE: corrected

DOCUMENT-IDENTIFIER: US 20030224360 A9

TITLE: Interventions to mimic the effects of calorie restriction

PUBLICATION-DATE: December 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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APPL-NO: 10/ 056749

DATE FILED: January 22, 2002

RELATED-US-APPL-DATA:

child 10056749 A9 20020122

parent continuation-of 09648642 20000825 US GRANTED

parent-patent 6406853 US

child 09648642 20000825 US

parent continuation-in-part-of 09471224 19991223 US ABANDONED

US-CL-CURRENT: 435/6, 435/4

ABSTRACT:

Long term calorie restriction has the benefit of increasing life span. Methods to screen interventions that mimic the effects of calorie restriction are disclosed. Extensive analysis of genes for which expression is statistically different between control and calorie restricted animals has demonstrated that specific genes are preferentially expressed during calorie restriction. Screening for interventions which produce the same expression profile will provide interventions that increase life span. In a further aspect, it has been discovered that test animals on a calorie restricted diet for a relatively short time have a similar gene expression profile to test animals which have been on a long term calorie restricted diet.

---------- KWIC ----------

Detail Description Table CWU - DETL (12):

10TABLE 10 mR:VAs decreased by age and returned to control levels by LT-CR GenBank Phenotype Immune System M30903 B lymphocyte kinase (Blk); src-family protein tyrosine kinase; plays important role in B-cell development/activation and immune responses; B-lineage cells U43384 Cytochrome b-245, beta polypeptide (Cybb, cytochrome b558); integral component of the microbicidal oxidase electron transport chain of phagocytic cells, respiratory burst oxidase; phagocytes U10871 Mitogen activated protein kinase 14 (Mapk14); signal transduction, stimulate phosphorylation of transcription

factors; major upstream activator of MAPKAP kinas 2; hematopoietic stem cells 222649 Myeloproliferative leukemia virus oncogene (Mpl); Member of hematopoietic cytokine receptor family, cell cycle regulator, induces proliferation and differentiation of hematopoietic cell lines; hematopoietic precursor cells, platelets and megakaryocytes Y07521 Potassium voltage gated channel, Shaw-related subfamily member 1 (Kcnc1) potassium channels with properties of delayed rectifiers; nervous system, skeletal system, T lymphocytes U87456 Flavin-containing monooxygenase 1 (Fmo1); xenobiotic metabolism; highly expressed in liver, lung, kidney, lower expressed in heart, spleen, testis, brain U40189 Pancreatic polypeptide receptor 1 (Ppyr1), neuropeptide Y receptor, peptide Y receptor; G-protein-coupled receptor; liver, gastrointestinal tract, prostate, neurons endocrine cells Neuron Specific U16297 Cytochrome b-561 (Cyb561); electron transfer protein unique to neuroendocrin secretory vesicles; vectoral transmembrane electron transport; brain D50032 Trans-golgi network protein 2 (Ttgn2); integral membrane protein localized to the trans-Golgi network; involved in the budding of exocytic transport vesicles; brain neurons Liver Specific/Ubiquitous D82019 Basigin (Bsg), CD147, neurothelin; membrane glycoprotein, immunoglobulin superfamily, homology to MHCs, acts as an adhesion molecule or a receptor, near: network formation and tumor progression; embryo, liver and other organs L38990 Glucokinase (Gk), key glycolytic enzyme; liver U50631 Heat-responsive protein 12 (Hrsp12); heat-responsive, phosphorylated protein sequence simularity to Hsp70; liver, kidney U39818 Tuberous sclerosis 2 (Tsc2); mutationally inactivated in some families with tuberoi sclerosis; encodes a large, membrane-associated GTPase activating protein (GA tuberlin); may have a key role in the regulation of cellular growth; ubiquitous

PGPUB-DOCUMENT-NUMBER: 20030195221

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030195221 A1

TITLE: Substituted indolizine-like compounds and methods of use

PUBLICATION-DATE: October 16, 2003

INVENTOR-INFORMATION:

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Dominguez, Celia	Thousand Oaks	CA	US	
Lu, Yuelie	Thousand Oaks	CA	US	
Rishton, Gilbert M.	Malibu	CA	US	

APPL-NO: 10/ 298205

DATE FILED: November 15, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60332447 20011116 US

US-CL-CURRENT: 514/258.1, 514/259.3 , 514/259.31 , 514/303 , 544/263 , 546/118

ABSTRACT:

Selected novel substituted indolizine-like compounds are effective for treatment of diseases, such as TNF-.alpha., IL-1.beta., IL-6 and/or IL-8 mediated diseases, and other maladies, such as cancer, pain and diabetes. The invention encompasses novel compounds, analogs, prodrugs and pharmaceutically acceptable salts thereof, pharmaceutical compositions and methods for treatment of diseases and other maladies or conditions involving inflammation, cancer, pain, diabetes and the like. The subject invention also relates to processes for making such compounds as well as to intermediates useful in such processes.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (16):

[0015] GB 2,306,108, which is incorporated herein by reference in its entirety, describes imidazole derivatives which are Raf kinase antagonists useful in the treatment of cancer which is mediated by Raf and Raf-inducible proteins. Raf proteins are kinases activated in response to extracellular mitogenic stimuli such as PDGF, EGF, acidic FGF, thrombin, insulin or endothelin, and also in response to oncoproteins such as v-src, v-sis, and v-fms. Raf functions downstream of ras in signal transduction from the cellular membrane to the nucleus. Compounds may be oncolytics through the antagonism of Raf kinase. It has been reported that antisense constructs which reduce cellular levels of c-Raf and hence Raf activity inhibit the growth of rodent fibroblasts in soft agar, while exhibiting little or no general cytotoxicity. This inhibition of growth in soft agar is highly predictive of tumor responsiveness in whole animals. Moreover, Raf antisense constructs have

shown efficacy in reducing tumor burden in animals. Examples of cancers where Raf kinase is implicated by overexpression include cancers of the brain, larynx, lung, lymphatic system, urinary tract and stomach, including hystocytic lymphoma, lung adenocarcinoma and small cell lung cancers. Other examples include cancers involving overexpression of upstream activators of Raf or Raf-activating oncogenes, including pancreatic and breast carcinoma.

PGPUB-DOCUMENT-NUMBER: 20030134302

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030134302 A1

TITLE: Libraries of expressible gene sequences

PUBLICATION-DATE: July 17, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 210985

DATE FILED: August 1, 2002

RELATED-US-APPL-DATA:

child 10210985 A1 20020801

parent continuation-of 10003021 20011114 US PENDING

child 10003021 20011114 US

parent continuation-of 09285386 19990402 US ABANDONED

non-provisional-of-provisional 60096981 19980818 US

non-provisional-of-provisional 60080626 19980403 US

US-CL-CURRENT: 435/6, 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

The invention described herein comprises libraries of expressible gene sequences. Such gene sequences are contained on plasmid vectors designed to endow the expressed proteins with a number of useful features such as affinity purification tags, epitope tags, and the like. The expression vectors containing such gene sequences can be used to transfect cells for the production of recombinant proteins. A further aspect of the invention comprises methods of identifying binding partners for the products of such expressible gene sequences.

RELATED APPLICATIONS

[0001] This application relies for priority on U.S. Provisional Application No. 60/080,626, filed Apr. 3, 1998, and U.S. Provisional Application No. 60/096,981, filed Aug. 18, 1998, each of which is hereby incorporated herein in its entirety.

---------- KWIC ---------

Detail Description Table CWU - DETL (19):

M221 E6 YLR142W proline oxidase (52.47/60) M84 C2 YLR144C Identified as an activity necessary for actin polymerization in permeabilized cells (85.72/90) M79 E4 YLR009W (22/32) M219 D4 YLR010C (17./6330) M219 D5 YLR011W (21.1/230) M219 D1 YLR015W (55.66/64) M219 D2 YLR016C (22.47/40) M219 D3 YLR017W Protein that regulates ADH2 gene expression (37.18/48) M219 E5 YLR019W (43.78/50) M219 E8 YLR022C (27.53/38) M80 A6 YLR026C Sed5p is a t-SNARE (soluble NSF attachment protein receptor) required in ER to Golgi transport. (37.43/25) M219 F5 YLR027C aspartate aminotransferase cytosolic (47.55/50) M79 F8 YLR029C Ribosomal protein RPL13A (YL10A) (rat L15) (22.47/30) M219 F8 YLR030W (29.04/40) M80 C2 YLR031W (20.57/32) M219 F3 YLR033W (55.33/55) M219 F6 YLR036C (22.46/33) M80 B10 YLR037C (13.67/13) M223 E1 YLR040C (24.67/38) M82 C6 YLR043C thioredoxin (11.46/12) M81 F7 YLR044C pyruvate decarboxylase (61.96/62) M82 D6 YLR051C (23.90/30) M222 G7 YLR053C (11.91/22) M82 C10 YLR054C (56.45/56) M223 B1 YLR055C transcription factor (66.35/70) M81 D2 YLR056W C-5 sterol desaturase (40.36/55) M81 H3 YLR057W (93.5/98) M81 D5 YLR058C serine hydroxymethyltransfe rase (51.62/55) M82 E6 YLR059C (29.62/30) M81 H7 YLR060W Phenylalanyl-tRNA synthetase alpha subunit cytoplasmic (65.56/65) M82 H8 YLR061W 402-755 (13.42/28) M222 A5 YLR066W signal peptidase subunit (20.45/34) M222 H3 YLR073C (22.03/34) M81 E5 YLR074C (18.39/28) M81 E5 YLR074C (18.39/28) M222 A6 YLR075W Ubiquinol- cytochrome C reductase complex subunit VI requiring protein (24.42/33) M222 A6 YLR075W Ubiquinol-cytochrome C reductase complex subunit VI requiring protein (24.42/33) M82 A8 YLR076C (15.43/16) M222 H7 YLR077W (64.24/67) M223 G5 YLR077W (64.24/60) M81 D1 YLR079W P40 inhibitor of Cdc28p-Clb5 protein kinase complex (31.45/50) M223 G3 YLR082C Smc4 protein member of SMC family (43.25/55) M222 B6 YLR083C integral membrane protein.backslash.p24a protein (73.40/90) M222 B4 YLR089C (65.25/65) M222 B4 YLR089C (65.25/65) M81 G5 YLR090W Homolog of E. coli DnaJ closely related to Ydj1p (50.6/60) M81 H6 YLR091W (32.34/40) M81 H6 YLR091W (32.34/40) M222 B8 YLR093C (27.64/39) M223 H2 YLR097C (37.87/52) M81 H5 YLR098C DNA binding activator (71.31/75) M222 D6 YLR099C (43.47/48) M82 C8 YLR100W (38.38/?) M82 C8 YLR100W (38.38/?) M81 A11 YLR102C (29.28/45) M82 F1 YLR103C omosomal DNA replication initiation protein (71.53/?) M222 E6 YLR107W (44.55/48) M222 D8 YLR109W (19.47/38) M81 G4 YLR113W mitogen-activated protein kinase (MAP kinase) (47.96/60) M81 A6 YLR114C (84.07/100) M81 A6 YLR114C (84.07/100) M223 D1 YLR119W suppressor of rnal-1 mutation (23.54/33) M222 D7 YLR124W (12.65/16) M222 F8 YLR125W (15.07/40) M82 A2 YLR127C APC (anaphase promoting complex) component (93.86/94) M82 D7 YLR131C activator of CUP1 expression (84.73/40) M222 E7 YLR132C (31.93/40) M221 A3 YLR137W (40.48/52) M84 C6 YLR139C (70.76/70) M86 G9 YLR141W Upstream activation factor subunit (40.04/55) M221 E6 YLR142W proline oxidase (52.47/60) M84 C2 YLR144C Identified as an activity necessary for actin polymerization in permeabilized cells (85.72/90) M221 F6 YLR150W (30.14/42) M255 H6 YLR151C (37.43/52) M84 G3 YLR153C acetyl-coenzyme A synthetase (75.16/75) M221 G4 YLR155C nitrogen catabolite-regulated cell-wall L- asparaginase II (39.85/50) M221 A2 YLR160C nitrogen catabolite- regulated cell-wall L- asparaginase II (39.85/50) M84 A8 YLR164W (18.59/19) M221 B1 YLR167W ubiquitin (16.83/16) M221 B2 YLR168C (25.33/35) M86 G8 YLR172C S-adenosylmethionine (AdoMet)-dependent methyltransferase of diphthamide biosynthesis (33.03/40) M224 F1 YLR175W major low affinity 55 kDa Centromere.backslash./microtub ule binding protein (53.24/60) M221 C2 YLR176C (89.24/96) M86 H5 YLR178C suppressor of cdc25 (24.12/38) M221 H4 YLR179C (22.14/33) M221 F5 YLR180W S-adenosylmethionine synthetase (42.13/48) M221 E4 YLR186W (27.83/36) M84 A7 YLR187W (112.97/114) M84 D8 YLR188W ATP-binding cassette (ABC) transporter family member (76.56/76) M84 H9 YLR189C (131.81/?) M84 D11 YLR190W (54.12/70) M84 G1 YLR191W Peroxisomal membrane protein that contains Src homology 3 (SH3) domain (42.57/45) M221 F3 YLR193C (19.38/30) M84 B7 YLR195C N-myristoyl transferase (50.08/32) M84 A10 YLR197W homology to

microtubule binding proteins and to X90565_5.cds (55.55/55) M221 D1 YLR199C (24.23/36) M221 E2 YLR200W Polypeptide 6 of a Yeast Non-native Actin Binding Complex homolog of a component of the bovine NABC complex (12.65/18) M84 D4 YLR201C (28.63/40) M84 C7 YLR203C Protein involved in maturation of COX1 and COB mRNA (47.99/48)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	174	hbx	US-PGPUB; USPAT	OR	OFF	2004/09/21 09:05
L2	492	(hepatitis adj b adj virus or hbv) near4 inhibit$8	US-PGPUB; USPAT	OR	OFF	2004/09/21 09:07
L3	26	1 and 2	US-PGPUB; USPAT	OR	OFF	2004/09/21 09:07
L4	11	hbx near4 inhibit$8	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:02
L5	10160	src	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:08
L6	794441	activat$8	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:08
L7	239593	upstream	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:09
L8	3912	6 near5 7	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:09
L9	27	8 same 5	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:13
L10	97	5 same 6 same 7	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:13

PGPUB-DOCUMENT-NUMBER: 20040152109

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040152109 A1

TITLE: Methods and compositions for determining risk of treatment toxicity

PUBLICATION-DATE: August 5, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Tibshirani, Robert	Stanford	CA	US	

APPL-NO: 10/ 686322

DATE FILED: October 14, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60419016 20021015 US

US-CL-CURRENT: 435/6

ABSTRACT:

Methods are provided for determining whether a patient treated with an anti-proliferative agent is susceptible to toxicity. In practicing the subject methods, an expression profile for the transcriptional response to a therapy is obtained from the patient and compared to a reference profile to determine whether the patient is susceptible to toxicity. In addition, reagents and kits thereof that find use in practicing the subject methods are provided.

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Detail Description Table CWU - DETL (6):

(rhabdomyosarcoma) UV 267 U03911 MSH2 mutS homolog 2 (colon cancer, nonpolyposis type 1) UV 268 AL021154 IR 269 AB011116 KIAA0544 KIAA0544 protein IR 270 X17644 GSPT1 G1 to S phase transition 1 UV 271 AI565760 GABARAPL2 GABA(A) receptor-associated protein-like 2 IR 272 D87735 RPL14 ribosomal protein L14 IR 273 U52112 IRAK1 interleukin-1 receptor-associated kinase 1 UV 274 X04803 ubiquitin IR 275 AI525834 NPC2 Niemann-Pick disease, type C2 gene IR 276 M14333 FYN FYN oncogene related to SRC, FGR, YES UV 277 Z97054 UREB1 upstream regulatory element binding protein 1 UV 278 AB014609 KIAA0709 endocytic receptor (macrophage mannose receptor UV family) 279 AI653621 TXN thioredoxin UV 280 U24266 ALDH4A1 aldehyde dehydrogenase 4 family, member A1 UV 281 M37583 H2AFZ H2A histone family, member Z UV 282 J03805 PPP2CB protein phosphatase 2 (formerly 2A), catalytic subunit, UV beta isoform 283 U51127 IRF5 interferon regulatory factor 5 UV 284 M22806 P4HB prolyl 4-hydroxylase beta-subunit and disulfide UV isomerase 285 D11086 IL2RG interleukin 2 receptor, gamma (severe combined UV immunodeficiency) 286

AF000982 DDX3 DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 3 UV 287 U86602 EBNA1BP2 EBNA1-binding protein 2 UV 288 AF000231 RAB11A RAB11A, member RAS oncogene family UV 289 L23959 TFDP1 transcription factor Dp-1 UV 290 AB020713 KIAA0906 KIAA0906 protein UV 291 X59871 TCF7 transcription factor 7 (T-cell specific, HMG-box) UV 292 AA310786 Homo sapiens cDNA: FLJ23602 fis, clone LNG15735 IR 293 U15085 HLA-DMB major histocompatibility complex, class II, DM beta IR 294 D80001 KIAA0179 KIAA0179 protein IR 295 HG4074-HT4344 Rad2 Rad2 UV 296 AA648295 CBX3 chromobox homolog 3 (HP1 gamma) UV 297 Y13936 PPM1G protein phosphatase IG (formerly 2C), magnesium- UV dependent, gamma isoform 298 D49489 P5 protein disulfide isomerase-related protein UV 299 AJ012590 H6PD hexose-6-phosphate dehydrogenase (glucose 1- IR dehydrogenase) 300 D16431 HDGF hepatoma-derived growth factor (high-mobility group IR protein 1-like) 301 AA527880 IR 302 AI525665 COX8 cytochrome c oxidase subunit VIII IR 303 U19765 ZNF9 zinc finger protein 9 (a cellular retroviral nucleic acid UV binding protein) 304 M74491 ARF3 ADP-ribosylation factor 3 UV 305 AF039397 UV 306 X67951 PRDX1 peroxiredoxin 1 IR 307 AB005047 SH3BP5 SH3-domain binding protein 5 (BTK-associated) IR 308 S75463 TUFM Tu translation elongation factor, mitochondrial UV 309 M63904 GNA15 guanine nucleotide binding protein (G protein), alpha UV 15 (Gq class) 310 D42084 METAP1 methionyl aminopeptidase 1 UV 311 W28979 FLJ20452 hypothetical protein FLJ20452 IR 312 M59465 TNFAIP3 tumor necrosis factor, alpha-induced protein 3 IR 313 M26004 CR2 complement component, receptor 2 IR 314 X04106 CAPNS1 calpain, small subunit 1 IR 315 Z14000 RING1 ring finger protein 1 UV 316 AF044671 GABARAP GABA(A) receptor-associated protein UV 317 D13627 CCT8 chaperonin containing TCP1, subunit 8 (theta) UV 318 D21853 KIAA0111 KIAA0111 gene product UV 319 HG662-HT662 Small Rna-Associated Protein IR 320 AI087268 SNRPC small nuclear ribonucleoprotein polypeptide C IR 321 D80000 SMC1L1 SMC1 (structural maintenance of chromosomes 1)- UV like 1 322 L31584 EBI 1 G protein-coupled receptor UV 323 M33336 PRKAR1A protein kinase, cAMP-dependent, regulatory, type I, alpha (tissue specific extinguisher 1) UV 324 D14812 KIAA0026 MORF-related gene X UV 325 D11139 TIMP1 tissue inhibitor of metalloproteinase 1 (erythroid UV potentiating activity, collagenase inhibitor) 326 M65028 HNRPAB heterogeneous nuclear ribonucleoprotein A/B UV 327 AB023154 KIAA0937 KIAA0937 protein UV 328 AA149486 COX17 COX17 homolog, cytochrome c oxidase assembly IR protein 329 Y00371 hsc70 71 kd heat shock cognate protein UV 330 X95808 ZNF261 zinc finger protein 261 IR 331 M64595 RAC2 ras-related C3 botulinum toxin substrate 2 (rho family, IR small GTP binding protein Rac2) 332 D50405 HDAC1 histone deacetylase 1 UV 333 X95384 UK114 translational inhibitor protein p14.5 UV 334 M93311 MT3 metallothionein 3 (growth inhibitory factor IR (neurotrophic)) 335 M13792 ADA adenosine deaminase UV 336 D90070 PMAIP1 phorbol-12-myristate-13-acetate-ind- uced protein 1 UV 337 AF047436 ATP5J2 ATP synthase, H+ transporting, mitochondrial F0 UV complex, subunit f, isoform 2 338 U24152 PAK1 p21/Cdc42/Rac1-activated kinase 1 (yeast Ste20- UV related) 339 U46692 cystatin B IR

PGPUB-DOCUMENT-NUMBER: 20040146903

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040146903 A1

TITLE: Methods and compositions for modulating activator protein 1

PUBLICATION-DATE: July 29, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Chanda, Sumit	La Jolla	CA	US	
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APPL-NO: 10/ 702112

DATE FILED: November 5, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60471864 20030520 US

non-provisional-of-provisional 60425344 20021107 US

US-CL-CURRENT: 435/6, 435/7.2 , 514/44

ABSTRACT:

This invention provides novel AP-1 modulatory polypeptides. The invention also provides methods for screening modulators of AP-1 transcription factor activities. The methods comprise first screening test agents for modulators of an AP-1-modulatory polypeptide and then further screening the identified modulating agents for modulators of AP-1 transcription factor activities. The invention further provides methods and pharmaceutical compositions for modulating AP-1 transcription factor activities in a cell and for treating diseases and conditions mediated by abnormal cellular proliferation.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Patent Application Serial Nos. 60/425,344 (filed Nov. 7, 2002) and 60/471,864 (filed May 20, 2003). The full disclosures of these applications are incorporated herein by reference in their entirety and for all purposes.

---------- KWIC ---------

Detail Description Paragraph - DETX (150):

[0163] To refine the mode of action by which the novel growth regulatory proteins are modulating the AP-1 pathway, their relative inductive activities were examined in the presence of small inhibitor RNA (siRNA) molecules directed against known components of AP-1 signaling. AP-1(PMA) reporter gene activities of pairwise cDNA (novel activators) and siRNA (AP-1 pathway members) transfections were normalized to appropriate controls and analyzed through

single-linkage hierarchical clustering (FIG. 2A). For comparison, effects of the inhibitory RNAs upon PMA-induced AP-1 activity are also shown. siRNAs encoding PI-3 kinase (PIK3R3, PIK3CD), PKC (PKCD, PRKACA, DGKI, MEKK2), and RAS (SRC, RAF) pathway members were most proficient in abrogating the AP-1 induction by the bulk of the identified growth regulators. This demarcates a majority of identified AP-1 activators as functioning upstream of these proteins, and suggests that they regulate AP-1 activity through modulation of PI-3 Kinase, PKC, and/or RAS pathways. For example, the results indicate that the activity of BLK upon the AP-1 reporter gene was extinguished by siRNAs coding for ERK pathway effectors DGKI, MEKK1, SRC, RAF1 and PIK3R3, but not by siRNAs directed against JNK-related pathway molecules MAP3K7, MAPK9, and MAP4K4. These observed activities are consistent with reports that these and related family members are important for AP-1 response to environmental growth stimuli.

PGPUB-DOCUMENT-NUMBER: 20040142859

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040142859 A1

TITLE: Method for treating neurodegenerative, psychiatric, and other disorders with deacetylase inhibitors

PUBLICATION-DATE: July 22, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Steffan, Joan S.	Laguna Beach	CA	US	
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Marsh, J. Lawrence	Newport Beach	CA	US	
Bodai, Laszlo	Irvine	CA	US	
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APPL-NO: 10/ 476627

DATE FILED: October 30, 2003

PCT-DATA:
APPL-NO: PCT/US02/14167
DATE-FILED: May 2, 2002
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 514/9, 514/557 , 514/568 , 514/575

ABSTRACT:

The invention relates to a novel method for treating a variety of diseases and disorders, including polyglutamine expansion diseases such as Huntington's disease, neurological degeneration, psychiatric disorders, and protein aggregation disorders and diseases, comprising administering to patients in need thereof of a therapeutically effective amount of one or more deacetylase inhibitors. The invention is also directed to a transgenic fly useful as a model of polyglutamine expansion diseases, which may be used to test potential therapeutic agents.

CROSS-REFERENCE TO RELATED APPLICATION(S)

[0001] This application claims the benefit of U.S. Provisional Application, Ser. No. 60/288,215, filed May 2, 2001 and entitled "Histone Deacetylase Inhibitors for Reducing Polyglutamine Toxicity in Vivo," the contents of which are hereby incorporated by reference in their entirety, and U.S. Provisional Application, Ser. No. _____, filed Apr. 11, 2002 and entitled "Treatment of Neurodegenerative, Psychiatric and other Disorders with Deacetylase Inhibitors," the contents of which are hereby incorporated by reference in their entirety.

---------- KWIC ---------

Detail Description Table CWU - DETL (1):

1 TABLE 1 Abbreviation Meaning AR Androgen receptor AT Acetyltransferase Br Bromodomain CBP CREB-binding protein CH Cysteine-histidine rich region DRPLA Dentatorubral-pallidoluysian atrophy GFP Green fluorescent protein GST Glutathione-S-transferase HAT Histone acetyltransferase HD Huntington's disease HDAC Histone deacetylase Htt Huntingtin Httex1p Huntingtin exon 1 protein KIX CREB-binding domain P/CAF p300/CBP-associated factor PMSF phenyl-methylsulfonyl fluoride Poly Q Polyglutamine RID Nuclear hormone receptor interacting domain RXR.alpha. Retinoid X receptor alpha SAHA Suberoylanilide hydroxamic acid SH3 Src homology 3 TBP TATA-binding protein TSA Trichostatin A UAS Upstream activator sequence

PGPUB-DOCUMENT-NUMBER: 20040142395

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040142395 A1

TITLE: Compositions and methods for identifying agents which modulate PTEN function and PI-3 kinase pathways

PUBLICATION-DATE: July 22, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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APPL-NO: 10/ 770725

DATE FILED: February 3, 2004

RELATED-US-APPL-DATA:

child 10770725 A1 20040203

parent continuation-of 09870379 20010530 US PENDING

non-provisional-of-provisional 60208437 20000530 US

non-provisional-of-provisional 60274167 20010308 US

US-CL-CURRENT: 435/7.23

ABSTRACT:

Methods are provided for the identification, biochemical characterization and therapeutic use of agents which impact PTEN, p53, PI-kinase and AKT mediated cellular signaling.

[0001] This invention claims priority under 35 U.S.C. .sctn.119(e) to U.S. Provisional Application Nos. 60/208,437 and 60/274,167 filed May 30, 2000 and Mar. 8, 2001 respectively. The entire disclosures of each of the above-identified applications is incorporated by reference herein.

---------- KWIC ---------

Detail Description Paragraph - DETX (141):

[0165] It is well known that Fc.gamma. receptor crosslinking induces the tyrosine phosphorylation of the adapter protein, Cbl (Park, R. K., et al., (1996) J. Immunology 160:5018). To determine if phagocytic signaling events lead to the phosphorylation of Cbl, the degree of Cbl phosphorylation was assessed before and after induction of phagocytosis. To investigate the role of specific kinases in this phosphorylation event, dominant negative Syk and the Src family kinase inhibitor PP1 were utilized to inhibit the activity of these enzymes. The results demonstrated that Cbl was phosphorylated on tyrosine residues following induction of phagocytosis and this phosphorylation event was abrogated by PP1 (FIGS. 11A and 11B, compare lanes 2-3 to 5-6). This

effect was dose dependent (data not shown), as was the effect of PP1 on inhibition of Fc.gamma. receptor-mediated phagocytosis (FIG. 11A). Interestingly, dominant negative Syk inhibited Cbl tyrosine phosphorylation to a lesser extent but completely abrogated the phagocytic response. Interestingly, both PP1 and dominant negative Syk suppressed the basal tyrosine phosphorylation levels of Cbl in vivo. These data suggested that the catalytic activity of the Src family kinases and the capacity of Syk to dock with the ITAM receptor were both required for Cbl phosphorylation in response to phagocytic stimuli and that these two events were required for phagocytosis. The dominant negative Syk would not be expected to alter the upstream activity of Src family kinases and hence Src mediated phosphorylation of Cbl was not altered to the same extent. The data provided support for a signaling cascade in which Syk functions downstream of Src and upstream of Cbl and other effectors associated with Cbl such as the p85 subunit of PI-3 kinase. The data demonstrated that Src family kinases mediated the phosphorylation of Cbl in a Syk kinase independent manner in vivo. The data also revealed that Src family kinases and Syk were required for phagocytosis mediated by the downstream activation of PI-3 kinase.

PGPUB-DOCUMENT-NUMBER: 20040138187

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040138187 A1

TITLE: Therapeutic treatment methods

PUBLICATION-DATE: July 15, 2004

INVENTOR-INFORMATION:

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Ahlem, Clarence N.	San Diego	CA	US	
Auci, Dominick L.	San Diego	CA	US	
Dowding, Charles	San Diego	CA	US	
Frincke, James M.	San Diego	CA	US	
Li, Mei	San Diego	CA	US	
Page, Theodore M.	Carlsbad	CA	US	
Stickney, Dwight R.	Granite Bay	CA	US	
Trauger, Richard J.	Leucadia	CA	US	
White, Steven K.	San Diego	CA	US	

APPL-NO: 10/ 651515

DATE FILED: August 28, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60407146 20020828 US

non-provisional-of-provisional 60408332 20020904 US

non-provisional-of-provisional 60479257 20030617 US

US-CL-CURRENT: 514/169

ABSTRACT:

The invention relates to the use of compounds to ameliorate or treat an condition such as a cystic fibrosis, neutropenia or other exemplified conditions. Exemplary compounds that can be used include 3.beta.-hydroxy-17.beta.-aminoandrost-5-ene, 3.beta.-hydroxy-16.alpha.-fluoro-17.beta.-aminoandrost-5-ene, 3.alpha.-hydroxy-16.alpha.-fluoro-17.beta.-aminoandrost-5-ene, 3.beta.-hydroxy-16.beta.-fluoro-17.beta.-aminoandrost-5-ene, 1.alpha.,3.beta.-dihydroxy-4.alpha.-fluoroandrost-5-ene-17-one, 1.alpha.,3.beta.,17.beta.-trihydroxy-4.alpha.-fluoroandrost-5-ene, 1.beta.,3.beta.-dihydroxy-6.alpha.-bromoandrost-5-ene, 1.alpha.-fluoro-3.beta.,12.alpha.-dihydroxyandrost-5-ene-17-one, 1.alpha.-fluoro-3.beta.,4.alpha.-dihydroxyandrost-5-ene and 4.alpha.-fluoro-3.beta.,6.alpha.,17.beta.-trihydroxyandrostane.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a nonprovisional application under 37 C.F.R. 1.53(b) and claims priority of pending U.S. provisional application Serial No. 60/407,146, filed Aug. 28, 2002, pending U.S. provisional application Ser. No. 60/408,332, filed Sep. 4, 2002, and U.S. provisional application Ser. No.

60/479,257, filed Jun. 17, 2003, all of which are incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (569):

[0567] The F1Cs can modulate the activity of certain biomolecules that mediate various biological responses that affect establishment or progression of a disease or that enhance or inhibit specific immune responses. Thus, in conditions where unwanted inflammation is present, the F1Cs can reduce inflammation, while enhancing Th1 or Tc1 immune responses at the same time. The biomolecules that the F1Cs can modulate include, e.g., transcription factors or receptors, including orphan nuclear receptors, and the homologs, isoforms, orthologs and co-factors (e.g., co-repressors, co-activators, transcription factors, gene promoter regions, sequences or messenger moieties such as calcium ions, potassium ions or cAMP) of any of these molecules and related molecules that participate in their function. The compounds can directly or indirectly from complexes with such molecules or they can modulate (detectably increase or decrease) the synthesis, level or one or more biological activities of those molecules. These complexes include receptors or transcription factor complexes, which can comprise heterodimers, homodimers and trimer, tetramer, pentamer or higher homo or hetero complexes. A number of the orphan receptors or their isoforms, orthologs or homologs, e.g., PPAR.alpha., PPAR.beta., PPAR.gamma., PPAR.gamma.1, PPAR.gamma.2, PPAR.gamma.3, LXR.alpha., LXR.beta., SXR, PXR, CAR.alpha. and CAR.beta., can form heterodimers with one or more of RXR.alpha., RX.beta., and RXR.gamma.. Exemplary mammalian, human or other biomolecules include steroidogenic factor-1 (SF-1), steroidogenic acute regulatory protein (StAR), a chicken ovalbumin upstream promoter-transcription factor (COUP-TF), chicken ovalbumin upstream promoter-transcription factor (COUP-TFI) and its mammalian isoforms, orthologs and homologs, silencing mediator for retinoid and thyroid hormone receptor (SMRT) and its mammalian isoforms, orthologs and homologs, sterol regulatory element binding protein (SREBP) 1a (SREBP-1a), SREBP-1c, SREPB-2, NF-E3, FKHR-L1, COUP-TFII and its mammalian isoforms, orthologs and homologs, and the isoforms, orthologs and homologs of I.kappa.B, I.kappa.BA, AML-3, PEBP2.alpha.A1, Osf2, Cbfa1, RUNX2, activating transcription factor 2 (ATF2), c-Jun, c-Fos, a mitogen activated kinase (MAP) such as p38 or JNK, a mitogen activated kinase kinase (MKK), a p160 or steroid receptor coactivator-1 family (SRC-1, SRC-1/serum response factor), SRC-2, SRC-3, SET, nerve growth factor inducible protein B, StF-IT, NFAT, NFAT interacting protein 45 (NIP45), IkB, an IkB kinase, NFATp, NFAT4, an AP-1 family protein, a p300 protein, CREB, CREB-binding protein (CPB), p300/CBP, p300/CPB-associated factor, SWI/SNF and their human and other homologs, BRG-1, OCT-1/OAF, AP-1, AF-2, Ets, androgen receptor associated protein 54 (ARA54), androgen receptor associated protein 55 (ARA55), androgen receptor associated protein 70 (ARA70), androgen receptor-interacting protein 3 (ARIP3), ARIP3/PIASx .alpha. complex, PIASx .alpha., Miz1, Miz1/PIASx .beta. complex, PIASx .beta., PIAS1, PIAS3, GBP, GBP/PIAS1 complex, RAC3/ACTR complex, SRC-1.alpha., receptor interacting protein-140 (RIP-140), transcription factor activator protein-1, activation function-2, glucocorticoid receptor-interacting protein-1 (GRIP-1), receptor interacting protein-160 (RIP-160), suppressor of gal4D lesions (SUG-1), transcription intermediary factor-1 (TIF-1), transcription intermediary factor-2 (TIF-2), SMRT, N-CoR, N-CoA-1, p/CIP, p65 (RelA), the 120 KD rel-related transcription factor, heat shock proteins (HSP) such as HSP90, HSP70 and HSP72, heat shock factor-1, Vpr encoded by the human immunodeficiency virus and its isoforms and homologs thereof, testicular orphan receptor 2 (TR2), testicular orphan receptor 4 (TR4), a thyroid hormone receptor .alpha., thyroid hormone receptor .alpha.1 (TR.alpha.1), thyroid hormone receptor .alpha.2 (TR.alpha.2), thyroid hormone receptor .beta.

(TR.beta.), retinoid X receptor .alpha. (RXR.alpha.), retinoid X receptor .beta. (RXR.beta.), retinoid X receptor .gamma. (RXR.gamma.), TR .alpha.1/RXR .alpha. heterodimer, direct repeat4 thyroid hormone response element (DR4-TRE), an estrogen receptor (ER) such as ER.alpha.or ER.beta., estrogen receptor related receptor .alpha. (ERR.alpha. or EER1), estrogen receptor related receptor .beta. (ERR.beta. or EER2), estrogen receptor related receptor .gamma. (ERR.gamma. or EER3), steroid xenobiotic receptor (SXR), a hepatocyte nuclear factor 4 (HNF4), hepatocyte nuclear factor 4.gamma. (HNF-4.gamma.), hepatocyte nuclear factor 3 (HNF-3), liver X receptors (LXRs), LXR.alpha., LXR.beta., estrogen receptor .alpha. (ER.alpha.), constitutive androstane receptor-.alpha. (CAR-.alpha.), constitutive androstane receptor-.beta. (CAR-.beta.), RXR/CAR-.beta. heterodimer, short heterodimer partner (SHP; NROB2), SHP/ER.alpha. heterodimer, estrogen receptor .beta., SHP/ER.beta. heterodimer, testicular orphan receptor TR4, TR2/TR4 heterodimer, pregnane X receptor (PXR) and isoforms, cytochrome P-450 monooxygenase 3A4, including its gene promoter region and isoforms thereof, HNF4/cytochrome P-450 monooxygenase 3A4 gene promoter region and isoforms complex, HIV-1 long terminal repeat (LTR), HIV-2 LTR, TR2/HIV-1 LTR complex, TR4/HIV-1 LTR complex, TR4/HIV-1 LTR complex, TR a1/TR4/HIV-1 LTR complex, TR2 isoforms (TR2-5, TR7, TR9, TR11), DAX-1 (NROB1), DAX-1/steroidogenic acute regulatory protein gene promoter region, RevErb, Rev-erbA .alpha., Rev-erb .beta., steroid receptor coactivator amplified in breast cancer (AIB 1), p300/CREB binding protein-interacting protein (p/CIP), thyroid hormone receptor (TR, T3R), thyroid hormone response elements (T3REs), retinoblastoma protien (Rb), tumor suppressor factor p53, transcription factor E2F, mammalian acute phase response factor (APRF), constitutive androstane receptor (CAR), Xehopus xSRC-3 and mammalian (e.g., human) isoforms, orthologs and homologs, TAK1, TAK1/peroxisome proliferator-activated receptor .alpha. (PPAR.alpha.) complex, PPAR.alpha./RXR.alpha. complex, peroxisome proliferator-activated receptor .beta. (PPAR.beta.), peroxisome proliferator-activated receptor .gamma. (PPAR.gamma.), peroxisome proliferator-activated receptor .delta. (PPAR.delta.), farnesoid X receptor, retina X receptor, TAK-1/RIP-140 complex, a retinoic acid receptor (RAR), retinoic acid receptor-.beta. (RAR.beta.), retinoic acid receptor-.gamma. (RAR.gamma.), TR4/RXRE complex, SF-1/steroid hydroxylase gene promoter region, SF-1/oxytocin, including its gene promoter region, a bile acid receptor (FXR), nuclear receptor corepressor (NcoR), liver receptor homologous protein-1 (LRH-1; NR5A2), SF-1/ACTH receptor gene promoter region, rat Ear-2 and mammalian homologs, human TR3 orphan receptor (TR3), RLD-1, OR-1, androgen receptor, glucocorticoid receptor, estrogen receptor, progesterone receptor, mineralcorticoid receptor, aldosterone receptor, E6-associated protein (E6-AP), OR1, OR1/RXR.alpha. complex, TIF-1, CBP/P300 complex, TRIP1/SUG-1 complex, RIP-140, steroid receptor coactivator 1 (SRC1), SRC1.alpha./P160 complex and TIF-2/GRIP-1 complex, RAR/N-CoR/RIP13 complex, RAR/SMRT/TRAC-2 complex and protein X of hepatitis B virus. The homologs, orthologs and isoforms of these transcription factors, receptors and other molecules are included among the molecules that the F1Cs can modulate the synthesis or one or more biological activities of. Such factors are biologically active or function in one or more of a number of cell types such as T cells, B cells, macrophages, dendritic cells, platelets, monocytes, neutrophils, neurons, epithelial cells, endothelial cells, cartilage cells, osteoblasts, osteoclasts, splenocytes, thymocytes and GALT associated cells. Methods to identify these molecules and their biological activities have been described, e.g., U.S. Pat. Nos. 6,248,781, 6,242,253, 6,180,681, 6,174,676, 6,090,561, 6,090,542, 6,074,850, 6,063,583, 6,051,373, 6,024,940, 5,989,810, 5,958,671, 5,925,657, 5,958,671, 5,844,082, 5,837,840, 5,770,581, 5,756,673, and PCT publication Nos. WO 00/24245, WO 0073453 and WO 97/39721.

PGPUB-DOCUMENT-NUMBER: 20040138161

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040138161 A1

TITLE: Methods of modulating proliferative conditions

PUBLICATION-DATE: July 15, 2004

INVENTOR-INFORMATION:

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Frank, Scott R.	Cambridge		US	

APPL-NO: 10/ 625486

DATE FILED: July 22, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60398088 20020724 US

US-CL-CURRENT: 514/44, 424/155.1 , 435/6 , 435/7.23 , 514/12

ABSTRACT:

A novel group of Myc-targets from the human genome is provided. Also provided are methods of making and using reagents for modulating activity of Myc-target gene products, and for the diagnosis and treatment of proliferative conditions that are regulated by Myc.

[0001] This application claims benefit from U.S. Provisional Patent Application No. 60/398,088, filed Jul. 24, 2002.

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Detail Description Table CWU - DETL (2):
gene, complete cds. -528 AF163763_p1 Elongation factor 1 A-2 (EF1A-2) gene, complete cds. 1411 AF163776_p1 TCF1 gene, partial cds. -1004 AF166335_p1 Integrin alpha 6 (ITGA6) gene, exon 1. -407 AF175325_p1 Eukaryotic initiation factor 4AI (EIF4A1) gene, partial cds -295 AF175325_p1 Eukaryotic initiation factor 4AI (EIF4A1) gene, partial cds 1125 AF187320 Transferrin receptor 559 AF196969_p1 Phenylalkylamine binding protein gene, complete cds; MG81 333 protein gene, partial cds; putative RNA-binding protein 3 RNP gene, complete cds; and MG21 pseudogene, complete sequence AF198614_p1 Mcl-1 (MCL-1) and Mcl-1 delta S/TM (MCL-1) genes, -579 alternative spliced forms, complete cds AF207550_p1 Protein translocase, JM26 protein, UDP-galactose translocator, -1587 pim-2 protooncogene homolog pim-2h, and shal-typ potassium channel genes, complete cds; JM12 protein an transcription factor IGHM enhancer 3 genes, partial cds. AF208234_p1 Cystatin B (CSTB) gene, promoter region and complete cds 11 AF208501_p1 Uncoupling protein 3 (UCP3) gene, promoter and exon 1. -1050 AF209746_p1 Beta tropomyosin (TPM2) gene, exons 1 through 8 and partial -762 cds AF223404 WNT1 inducible signaling pathway

protein 1 (WISP1) gene, -4602 promoter and partial cds AF223404_p1 WNT1 inducible signaling pathway protein 1 (WISP1) gene, -1101 promoter and partial cds AF224272_p1 Cyclin dependent kinase 4, promoter region. -125 AF224272_p1 Cyclin dependent kinase 4, promoter region. -198 U37022_p1, cdk4 -29 AF224272_p1 U37022_p1, cdk4 455 AF224272_p1 AF239710_p1 DNA polymerase delta small subunit (POLD2) gene, exons 1 287 through 11 and complete cds AF255613_p1 Teratoma-associated tyrosine kinase (TAPK) gene, exons 1 349 through 6 and partial cds AF257772 RNA binding protein MCG10 gene -2257 AF258623_p1 ATP binding cassette transporter 1 (ABCA1) gene, promoter -64 and exon 1 AF258674_p1 MUCDHL (MUCDHL) gene, complete cds, alternatively -469 spliced AF270493 Id2 -1590 AF289220 BCL2L12 65 AF293386_p1 Eukaryotic translation initiation factor 5AII (EIF5A2) gene, -186 exons 1, 2, and 3 AJ001686_p1 NKG2F gene. -1322 AJ002311_i1 Synaptogyrin 2 intron 1 AJ006239.sub.-- QDPR; dihydropteridine reductase intron 1 AJ009866_p1 pex3 gene (joined CDS, promoter and exon 1). -1086 AJ009866_p1 pex3 gene (joined CDS, promoter and exon 1). -752 AJ010341_p1 PISSLRE gene, exons 1, 2, and 3 and joined CDS. 779 AJ010395_i1 DKC1 680 AJ010395_i1 DKC1 653 AJ011802_p1 OZF gene exon 1. -676 AJ011802_p1 OZF gene exon 1. 27 AJ012453_p1 MUC5B gene proximal 5' flanking region. 1027 AJ131016_p1 SCL gene locus. -147 AJ131757 olr1 2481 AJ131612 dic 1749 AJ224639_p1 Surf-5 and Surf-6 genes. -368 AJ224639_p1 Surf-5 and Surf-6 genes. -219 aj238481_p1 FBP2 gene fructose-1,6-bisphosphatase 2 -1450 AJ238482_i1 FBP2; fructose-1,6-bisphosphatase 2 1291 AJ238511_i1 MVP intron 1 aj238592 SLAP (src-like adaptor protein) -2974 AJ243297_i1 RET -837 AJ245489_p1 GCGR gene for glucagon receptor, promoter I. -1100 AJ249162 Enhancer from ISG20 AJ249275_p2 Partial MTHFR gene for methylenetetrahydrofolate reductase -1108 AJ249275_p2 Partial MTHFR gene for methylenetetrahydrofolate reductase -797 AJ249275_p2 Partial MTHFR gene for methylenetetrahydrofolate reductase -774 AJ250235_i1 FECH gene for ferrochelatase 5944 AJ250249_p1 Partial Mif1/KIAA0025 gene, 5'-upstream region. -475 AJ250249_p1 Partial Mif1/KIAA0025 gene, 5'-upstream region. -168 AJ250915_p1 p10 gene for chaperonin 10 (Hsp10 protein) and p60 gene for 232 chaperonin 60 (Hsp60 protein) AJ250915_p1 p10 gene for chaperonin 10 (Hsp10 protein) and p60 gene for -522 chaperonin 60 (Hsp60 protein) AJ272029_p1 Partial CD30 gene for cytokine receptor CD30 and promoter 951 region AL021154 Id3 -2895 AL022312_p2 ATF4 gene -86 AL121928_p1 DNA sequence from clone RP11-18I14, gene bA18I14.4 -1514 AL133551_p1 SIRT1 gene (Sir2-like proteins (siruitins) type 1) -1049 AL133551_p1 SIRT1 gene (Sir2-like proteins (siruitins) type 1) -423 D00591_p1 RCC1 gene, exons 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 1055 complete cds D13156_p1 Elafin, complete cds. 343 D13370_p1 APX gene encoding APEX nuclease, complete cds. -9 D14668_p1 Proteasome HC5, 5'-flanking region. 47 D17616_p1 N-acetylgalactosamine 6-sulfate sulfatase (GALNS), exon 1 -187 D21801_p1 Proteasome subunit HC8, 5'-flanking region. -660 D28877_i1 hnRNP protein A2/B1 2218 D38592_i1 MTH1 gene for 8-oxo-dGTPase -56 D50030_p1 Hepatocyte growth factor activator, complete cds 1873 D63395_p1 NOTCH4, partial cds. 870 D63861_p1 Cyclophilin 40, complete cds. 499 D63861_p1 Cyclophilin 40, complete cds. 949 D85429_p1 Heat shock protein 40, complete cds. -1346 D85922_i1 single-minded 2 177 D85922_i1 single-minded 2 -360 D87675_p1 Amyloid precursor protein, complete cds. 3267 D87675_p1 Amyloid precursor protein, complete cds. 568 D87943_i1 Alpha(1,2)fucosyltransferase intron 1 D90084_p1 Pyruvate dehydrogenase(EC 1.2.4.1)alpha subunit gene, exons 1-11 826 J00153_p1 Alpha globin psi-alpha-1, alpha-2 and alpha-1 genes, complete 371 cds J03466_p1 Insulin receptor gene, exon 1, clone p-lambda EA2. -876 J03764_p1 Plasminogen activator inhibitor-1 gene, exons 2 to 9. -556 J03764_p1 Plasminogen activator inhibitor-1 gene, exons 2 to 9. -440 J04038_p1 Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene, 666 complete cds J04111_p1 c-jun proto oncogene (JUN), complete cds, clone hCJ-1. 946 J04201_p1 Beta-polymerase gene, exons 1 and 2. -525 J04809_p1 Cytosolic adenylate kinase (AK1) gene, complete cds. -818 J04988_p1 90 kD heat shock

protein gene, complete cds. 374 J05096_p1 Na, K-ATPase subunit alpha 2 (ATP1A2) gene, complete cds. 1228 J05253_i1 Interstitial retinol-binding prot. prec. (IRBP) 4328 K02402_i1 Coagulation factor IX 2866 K03014_p1 MHC class II HLA-SB-beta-1 gene (untyped), clone LC11. 1564 K03021_p1 Tissue plasminogen activator (PLAT) gene, complete cds. 557 L04147_p1 Neurofilament light chain (NEFL) gene, promoter region. -1239 L06162_p1 Breast cancer-associated antigen (DF3) gene, 5' end and -1583 promoter region L06484_p1 Acetylcholinesterase (ACHE) gene, exons 1-2, and promoter -262 region L07485_p1 Deoxycytidine kinase gene, promoter region. -450 L10137_p1 Histone (H2AZ) gene, promoter sequence. -392 L10347_i1 Pro-alphal type II collagen (COL2A1) 1219 L10822_p1 Gastrin receptor gene, complete cds. 1872 L11016_p1 Lymphotoxin-beta gene, complete cds. -1531 L12399_p1 Nuclear lamin A and nuclear lamin C gene, exon 1. -707 L13391_p1 Helix-loop-helix basic phosphoprotein (G0S8) gene, complete -823 cds L14272_p1 Prohibitin (PHB) gene, exons 1-7. -352 L16842_p1 Ubiquinol cytochrome-c reductase core I protein mRNA, 883 complete cds L19314 HRY (Hairy, or HHL, HES-1) -1260 L21905_p1 Troponin I, slow-twitch isoform(TNNI1) gene, exon 1. -567 L22298_p1 Moesin gene. -335 L24442_p1 Interferon regulatory factor 2 (IRF2) gene, 5' flank. -403 L25932_p1 Integral nuclear envelope inner membrane protein (LBR) gene, 1019 5' UTR, exons 1 and 2 127148 Galanin gene, 5' end. -2487 L27148_p1 Galanin gene, 5' end. -1383 L27587_i1 CD79b/Ig beta/B29 -644 L29530_i1 CACNL1A1 calcium channel L-type alpha 1 sub. -129 L29766_i1 Epoxide hydrolase (EPHX1) -2207 X77738_p1 Red cell anion exchanger (EPB3, AE1, Band 3) gene, 3' region -940 L39891_i1 Polycystic kidney disease-associated protein (PKD1) 10910 L41560_i1 PCBD; pterin-4a-carbinolamine dehydratase -4219 L41919_p1 Candidate tumor suppressor HIC-1 (HIC-1) gene, complete cds 1338 L44140_p1 Chromosome X region from filamin (FLN) gene to glucose-6- 1176 phosphate dehydrogenase (G6PD) gene, complete

PGPUB-DOCUMENT-NUMBER: 20040106184

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040106184 A1

TITLE: Chromatographic methods for adenovirus purification

PUBLICATION-DATE: June 3, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 649974

DATE FILED: August 27, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60406591 20020828 US

US-CL-CURRENT: 435/239

ABSTRACT:

This invention provides methods for passing adenovirus particle preparations through chromatographic media to provide purified adenovirus particles.

[0001] This application is an International application, claiming benefit to U.S. Provisional Patent Application No. 60/406,592 filed Aug. 28, 2002. The entire disclosure of the foregoing application is incorporated herein by reference.

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Detail Description Table CWU - DETL (4):
4TABLE A Transgenes Gene Source Human Disease Function Growth Factors HST/KS Transfection FGF family members INT-2 MMTV promoter FGF family members insertion INTI/WNTI MMTV promoter Factor-like insertion SIS Simian sarcoma virus PDGF B Receptor Tyrosine Kinases ERBB/HER Avian erythroblastosis Amplified, deleted EGF/TGF- virus; ALV promoter squamous cell .alpha./amphiregulin/ insertion; amplified cancer; glioblastoma hetacellulin receptor human tumors ERBB- Transfected from rat Amplified breast, Regulated by 2/NEU/HER-2 glioblastomas ovarian, gastric NDF/heregulin and cancers EGF-related factors FMS SM feline sarcoma CSF-1 receptor virus KIT HZ feline sarcoma MGF/Steel receptor virus hematopoiesis MET Transfection from Scatter factor/HGF human osteosarcoma receptor PDGF receptor Translocation Chronic TEL (ETS-like myclomonocytic transcription leukemia factor)/PDGF receptor gene fusion RET Translocations and Sporadic thyroid Orphan receptor Tyr point mutations cancer; familial kinase medullary thyroid cancer; multiple endocrine neoplasias 2A and 2B ROS URII avian sarcoma Orphan receptor Tyr virus kinase TGF-.beta. receptor Colon carcinoma mismatch mutation target TRK Transfection from NGF (nerve growth human colon cancer factor) receptor Non-Receptor Tyrosine Kinases ABL Abelson Mul. V Chronic

Interact with RB, RNA, myelogenous polymerase, CRK, CBL leukemia translocation with BCR FPS/FES Avian Fujinami SV; GA FeSV LCK Mul. V (murine Src family; T-cell leukemia virus) signaling; interacts promoter CD4/CD8 T-cells SRC Avain Rous sarcoma Membrane-associated virus Tyr kinase with signaling function; activated by receptor kinases YES Avian Y73 virus Src family; signaling SER/THR Protein Kinases AKT AKT8 murine Regulated by PI3K; retrovirus regulate 70-kd S6 MOS Maloney murine SV GVBD; cytostatic factor; MAP kinase kinase PIM-1 Promoter insertion mouse RAF/MIL 3611 murine SV; Signaling in RAS MH2 avian SV pathway Miscellaneous Cell Surface APC Tumor suppressor Colon cancer Interacts with catenin DCC Tumor suppressor Colon cancer CAM domains E-cadherin Candidate for tumor Breast cancer Extracellular homotypic suppressor binding; intracellular interacts with catenins PTC/NBCCS Tumor suppressor and Nevoid basal cell 12 transmembrane Drosophila homology cancer syndrome domain; signals through (Gorline syndrome) Gli homologue Cl to antagonize hedgehog pathway Tan-1 Notch Translocation T-ALL Signaling? homologue Miscellaneous Signaling BCL-2 Translocation B-cell lymphoma Apoptosis CBL Mu Cas NS-1 V Tyrosine- phosphorylated RING finger interact Ab1 CRK CT1010 ASV Adapted SH2/SH3 interact Ab1 DPC4 Tumor suppressor Pancreatic cancer TGF-.beta. related signaling pathway MAS Transfection and Possible angiotensin tumorigenicity receptor NCK Adaptor SH2/SH3 SSeCKS protein kinase C substrate with tumor suppressor activity Guanine Nucleotide Exchangers and Binding Proteins BCR Translocated with Exchanger; protein ABL and CML kinase DBL Transfection Exchanger GSP NF-1 Hereditary tumor Tumor suppressor RASS GAP suppressor neurofibromatosis OST Transfection Exchanger Harvey-Kirsten, HaRat SV; Ki RaSV; Point mutations in Signal cascade N-RAS Balb-MoMuSV; many human tumors Transfection VAV Transfection S112/S113; Exchanger Nuclear Proteins and Transcription Factors BRCA1 Heritable suppressor Mammary/ovarian Localization unsettled cancer BRCA2 Heritable suppressor Mammary cancer Function unknown C/EBP.alpha. ERBA Avian erythroblastosis Thyroid hormone virus receptor (transcription) ETS Avian E26 virus DNA binding EVII MuLV promoter AML Transcription factor insertion FOS FBI/FBR murine 1 transcription factor osteosarcoma viruses with c-JUN GLI Amplified glioma Glioma Zinc finger; cubitus interruptus homologue is in hedgehog signaling pathway; inhibitory link PTC and hedgehog GSP Oncogene I.kappa.B HMGG/LIM Trasnlocation t(3:12) Lipoma Gene fusions high t(12:15) mobility group HMGI- C (XT-hook) and transcription factor LIM or acidic domain JUN ASV-17 Transcription factor AP-1 with FOS MLL/VHRX + ELI/ Translocation/fusion Acute myeloid Gene fusion of DNA- MEN ELL with MLL leukemia binding and methyl trithorax-like gene transferase MLL with ELI RNA pol II elongation factor MYB Avian myeloblastosis DNA binding virus MYC Avian MC29; Burkitt's lymphoma DNA binding with translocation B-cell MAX partner; cyclin lymphomas; promoter regulation; interact RB; insertion avian regulate apoptosis leucosis virus N-MYC Amplified Neuroblastoma L-MYC Lung cancer NEU Oncogene NF.kappa.B Par-4 RAF Oncogene RAS Oncogene REL Avian NF-kB family retriculoendotheliosis transcription factor SKI Avian SKV770 Transcription factor retrovirus VHL Heritable suppressor Von Hippel-Landau Negative regulator or syndrome elongin; transcriptional elongation complex WT-1 Wilm's tumor Transcription factor Cell Cycle/DNA Damage Response 53BP2 Tumor suppressor APC Tumor suppressor ATM Hereditary disorder Ataxia-telangiectasia Protein/lipid kinase homology; DNA damage response upstream in p53 pathway BAP1 Tumor suppressor protein involved in cell cycle control BCL-2 Translocation Follicular lymphoma Apoptosis C-CAM Plasma membrane of Tumor suppressor rate hepatocytes CDK Regulates transitions through the cell cycle DBCCR1 DCC Tumor suppressor DPC-4 Tumor suppressor E2F-1 E2F-2 E2F-3 E2F-4 FACC Point mutation Fanconi's anemia group C (predisposition leukemia) FCC FHIT Fragile site 3p14.2 Lung carcinoma Histidine triad-related diadenosine 5',3'-P.sup.1.p.sup.4 tetraphosphate asymmetric hydrolase Flt1 (soluble) anti-angiogenic hemopexin anti-angiogenic

PGPUB-DOCUMENT-NUMBER: 20040106156

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040106156 A1

TITLE: Methods and compositions for detecting receptor-ligand interactions in single cells

PUBLICATION-DATE: June 3, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 346620

DATE FILED: January 16, 2003

RELATED-US-APPL-DATA:

child 10346620 A1 20030116

parent continuation-in-part-of 10193462 20020710 US PENDING

non-provisional-of-provisional 60304434 20010710 US

non-provisional-of-provisional 60310141 20010802 US

US-CL-CURRENT: 435/7.2

ABSTRACT:

The invention provides methods and compositions for simultaneously detecting the activation state of a plurality of proteins in single cells using flow cytometry. The invention further provides methods and compositions of screening for bioactive agents capable of coordinately modulating the activity of a plurality of proteins in single cells. The methods and compositions can be used to determine the protein activation profile of a cell for predicting or diagnosing a disease state, and for monitoring treatment of a disease state.

[0001] This application is a continuation in part of U.S. Ser. No. 10/193,462, filed Jul. 10, 2002, which claims the benefit of the filing date of U.S. Ser. No. 60/304,434, filed Jul. 10, 2001, and U.S. Ser. No. 60/310,141, filed Aug. 2, 2001.

---------- KWIC ---------

Detail Description Paragraph - DETX (206):

[0233] The present inventors undertook flow cytometric based p44/42 MAPK kinase inhibition and activation profiling to identify necessary components for LFA-1 signaling. PKC inhibitor BIM I, cytoskeletal disrupting agents cytochalisin D, taxol, nocodozole, and sequestering of divalent cations by EDTA diminished the ICAM-2 induced p44/42 MAPK signal (FIG. 3D), suggesting that the

ligand-induced events of LFA-1 are mechanically linked to signal transduction by the actin-microtubule cytoskeleton. To identify upstream kinases that were responsible for signal transmission from LFA-1 to p44/42 MAPK, a series of kinase inhibitors were applied and tested for their ability to abrogate the ICAM-2 induced p44/42 MAPK activity (FIGS. 2H-I), whereas Herbimycin A and Emodin, inhibitors of src and p56lck had no effect. Tyrphostin A9 and piceatannol, specific inhibitors of proline-tyrosine kinase 2 (Pyk2) and Spleen-tyrosine kinase (Syk), respectively (Avdi, et al. (2001) J Biol Chem 276, 2189-2199.; Fuortes, et al., (1999) J Clin Invest 104, 327-335) abrogated the ICAM-2 induced activation of p44/42 MAPK and its upstream activator Raf-1 (FIG. 4A).

PGPUB-DOCUMENT-NUMBER: 20040102425

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040102425 A1

TITLE: Selective glucocorticoid receptor agonists

PUBLICATION-DATE: May 27, 2004

INVENTOR-INFORMATION:

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Miel, Hughes Jean-Pierre	Belfast		GB	
Ray, David William	Manchester		GB	
Brass, Andrew Michael	Manchester		GB	

APPL-NO: 10/ 415711

DATE FILED: December 5, 2003

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
GB	0026947.2	2000GB-0026947.2	November 3, 2000
GB	0115161.2	2001GB-0115161.2	June 20, 2001

PCT-DATA:
APPL-NO: PCT/GB01/04888
DATE-FILED: Nov 2, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 514/173, 514/179

ABSTRACT:

This invention is directed to a method for treating an inflammatory condition, treating haematological and other malignancies, causing immunosuppression, or preventing or treating transplant rejection in man or other animals which comprises administering to a patient a compound that has the structure of Formula (I) or Formula (II) as defined below, or a pharmaceutically acceptable derivative thereof or pro-drug therefor, wherein R=NH.sub.2, NHR.sup.1, NHOR.sup.2, NHNHR.sup.2, NHCOR.sup.2, and R.sup.1=C.sub.(1-4)alkyl, C.sub.(3-6)cycloalkyl, C.sub.n, where n=1-3, R.sub.2=methyl, ethyl, R.sub.3=alkyl, cycloalkyl, substituted alkyl, substituted cycloalkyl, aryl, heteroaryl, substituted aryl, or substituted hetreoaryl; wherein R.sup.4, R.sup.5=C.sub.(1-4)alkyl. Novel compounds according to Formula (III), wherein R.sup.6 and R.sup.7 are any of H, CH.sub.3CO, CH.sub.3CH.sub.2CO, CH.sub.3CH.sub.2CH.sub.2CO provided that R.sup.6 and R.sup.7 are not both H, or Formula (IV), wherein R.sup.8 and R.sup.9 are any of H, CH.sub.3CO, CH.sub.3CH.sub.2CO or CH.sub.3CH.sub.2CH.sub.2CO, having use in such methods, are also described.

--------- KWIC ---------

Detail Description Paragraph - DETX (46):

[0082] SRC-1 is an important co-activator of GR action that associates with the GR in a ligand dependant manner. The ability of SRC-1 to associate with the GR in this manner can be used as a measure of potency of ligand mediated transactivation. To perform this assay plasmids that generated VP16-GR ligand binding domain (LBD) and GAL4-SRC-1 fusion proteins and a GAL4 binding luciferase reporter gene construct were transfected into in a cell line lacking GR and deficient in endogenous SRC-1. The GAL4SRC-1 fusion protein could bind to specific sites upstream of the luciferase reporter gene construct. If the SRC-1 fusion protein associated with the ligand bound VP16-GRLBD fusion protein then the VP-16 activation domain augmented the luciferase gene expression. Ligand dose responses were analysed for SRC-1 binding for the compounds shown in FIGS. 1 and 2A.

PGPUB-DOCUMENT-NUMBER: 20040086485

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040086485 A1

TITLE: Chemeric viral vectors for gene therapy

PUBLICATION-DATE: May 6, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Aguilar-Cordova, Carlos Estuardo	Newton	MA	US	

APPL-NO: 10/ 264839

DATE FILED: October 4, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60327179 20011004 US

US-CL-CURRENT: 424/93.2, 435/456

ABSTRACT:

A nucleic acid sequence in a plasmid form comprising all the necessary elements for the production of a viral vector and this plasmid is delivered in-vivo with the intent of in-vivo viral vector production. The delivery of this vector may be further directed to specific targeted tissues by the addition of conjugated molecules, such as polycations, peptides, antibodies, single chain antibodies or combinations of the above.

---------- KWIC ---------

Summary of Invention - Table CWU - BSTL (3):

3TABLE 3 Oncogenes Gene Source Human Disease Function Growth Factors.sup.1 FGF family member HST/KS Transfection INT-2 MMTV promoter FGF family member Insertion INTI/WNTI MMTV promoter Factor-like Insertion SIS Simian sarcoma virus PDGF B Receptor Tyrosine Kinases.sup.1,2 ERBB/HER Avian erythroblastosis Amplified, deleted EGF/TGF-.alpha./ virus; ALV promoter squamous cell amphiregulin/ insertion; amplified cancer; glioblastoma hetacellulin receptor human tumors ERBB-2/NEU/HER-2 Transfected from rat Amplified breast, Regulated by NDF/ Glioblatoms ovarian, gastric cancers heregulin and EGF- related factors FMS SM feline sarcoma virus CSF-1 receptor KIT HZ feline sarcoma virus MGF/Steel receptor hematopoieis TRK Transfection from NGF (nerve growth human colon cancer factor) receptor MET Transfection from Scatter factor/HGF human osteosarcoma receptor RET Translocations and point Sporadic thyroid cancer; Orphan receptor Tyr mutations familial medullary kinase thyroid cancer; multiple endocrine neoplasias 2A and 2B ROS URII avian sarcoma Orphan receptor Tyr Virus kinase PDGF receptor Translocation Chronic TEL(ETS-like Myclomonocytic transcription factor)/ Leukemia PDGF receptor gene fusion TGF-.beta. receptor Colon carcinoma mismatch mutation target NONRECEPTOR TYROSINE KINASES.sup.1 ABI. Abelson Mul.V Chronic myelogenous Interact with RB, RNA leukemia translocation polymerase, CRK, with BCR CBL FPS/FES Avian Fujinami SV; GA

FeSV LCK Mul.V (murine leukemia Src family; T cell virus) promoter insertion signaling; interacts CD4/CD8 T cells SRC Avian Rous sarcoma Membrane-associated Virus Tyr kinase with signaling function; activated by receptor kinases YES Avian Y73 virus Src family; signaling SER/THR PROTEIN KINASES.sup.1 AKT AKT8 murine retrovirus Regulated by PI(3)K?; regulate 70-kd S6 k? MOS Maloney murine SV GVBD; cystostatic factor; MAP kinase kinase PIM-1 Promoter insertion Mouse RAF/MIL 3611 murine SV; MH2 Signaling in RAS avian SV pathway MISCELLANEOUS CELL SURFACE.sup.1 APC Tumor suppressor Colon cancer Interacts with catenins DCC Tumor suppressor Colon cancer CAM domains E-cadherin Candidate tumor Breast cancer Extracellular homotypic Suppressor binding; intracellular interacts with catenins PTC/NBCCS Tumor suppressor and Nevoid basal cell cancer 12 transmembrane Drosophilia homology syndrome (Gorline domain; signals syndrome) through Gli homogue CI to antagonize hedgehog pathway T A N -1 Notch Translocation T-ALI. Signaling? homologue MISCELLANEOUS SIGNALING.sup.1,3 BCL-2 Translocation B-cell lymphoma Apoptosis CBL Mu Cas NS-1 V Tyrosine-phosphorylated RING finger interact Abl CRK CT1010 ASV Adapted SH2/SH3 interact Abl DPC4 Tumor suppressor Pancreatic cancer TGF-.beta.-related signaling pathway MAS Transfection and Possible angiotensin Tumorigenicity receptor NCK Adaptor SH2/SH3 GUANINE NUCLEOTIDE EXCHANGERS AND BINDING PROTEINS.sup.3,4 BCR Translocated with ABL Exchanger; protein in CML kinase DBL Transfection Exchanger GSP NF-1 Hereditary tumor Tumor suppressor RASGAP Suppressor Neurofibromatosis OST Transfection Exchanger Harvey-Kirsten, N-RAS HaRat SV; Ki RaSV; Point mutations in many Signal cascade Balb-MoMuSV; human tumors Transfection VAV Transfection S112/S113; exchanger NUCLEAR PROTEINS AND TRANSCRIPTION FACTORS.sup.1,5-9 BRCA1 Heritable suppressor Mammary Localization unsettled cancer/ovarian cancer BRCA2 Heritable suppressor Mammary cancer Function unknown ERBA Avian erythroblastosis thyroid hormone Virus receptor (transcription) ETS Avian E26 virus DNA binding EVII MuLV promoter AML Transcription factor Insertion FOS FBI/FBR murine 1 transcription factor osteosarcoma viruses with c-JUN GLI Amplified glioma Glioma Zinc finger; cubitus interruptus homologue is in hedgehog signaling pathway; inhibitory link PTC and hedgehog HMGG/LIM Translocation t(3:12) Lipoma Gene fusions high t(12:15) mobility group HMGI-C (XT-hook) and transcription factor LIM or acidic domain JUN ASV-17 Transcription factor AP-1 with FOS MLL/VHRX + ELI/MEN Translocation/fusion Acute myeloid leukemia Gene fusion of DNA- ELL with MLL binding and methyl Trithorax-like gene transferase MLL with ELI RNA pol II elongation factor MYB Avian myeloblastosis DNA binding Virus MYC Avian MC29; Burkitt's lymphoma DNA binding with Translocation B-cell MAX partner; cyclin Lymphomas; promoter regulation; interact Insertion avian RB?; regulate leukosis apoptosis? Virus N-MYC Amplified Neuroblastoma L-MYC Lung cancer REL Avian NF-.kappa.B family Retriculoendotheliosis transcription factor Virus SKI Avian SKV770 Transcription factor Retrovirus VHL Heritable suppressor Von Hippel-Landau Negative regulator or Syndrome elongin; transcriptional elongation complex WT-1 Wilm's tumor Transcription factor CELL CYCLE/DNA DAMAGE RESPONSE.sup.10-21 ATM Hereditary disorder Ataxia-telangiectasia Protein/lipid kinase homology; DNA damage response upstream in P53 pathway BCL-2 Translocation Follicular lymphoma Apoptosis FACC Point mutation Fanconi's anemia group C (predisposition Leukemia FHIT Fragile site 3p14.2 Lung carcinoma Histidine triad-related diadenosine 5',3""- P.sup.1.p.sup.4 tetraphosphate asymmetric hydrolase hMLI/MutL HNPCC Mismatch repair; MutL homologue hMSH2/MutS HNPCC Mismatch repair; MutS homologue hPMS1 HNPCC Mismatch repair; MutL homologue hPMS2 HNPCC Mismatch repair; MutL homologue INK4/MTS1 Adjacent INK-4B at Candidate MTS1 p16 CDK inhibitor 9p21; CDK complexes Suppressor and MLM Melanoma gene INK4B/MTS2 Candidate suppressor p15 CDK inhibitor MDM-2 Amplified Sarcoma Negative regulator p53 p53 Association with SV40 Mutated >50% human Transcription factor; T antigen tumors, including checkpoint control; hereditary Li-Fraumeni apoptosis syndrome PRAD1/BCL1 Translocation with Parathyroid

adenoma; Cyclin D Parathyroid hormone B-CLL or IgG RB Hereditary Retinoblastoma; Interact cyclin/cdk; Retinoblastoma; Osteosarcoma; breast regulate E2F Association with many DNA virus tumor cancer; other sporadic cancers transcription factor Antigens XPA Xeroderma Excision repair; photo-Pigmentosum; skin product recognition; cancer predisposition zinc finger

PGPUB-DOCUMENT-NUMBER: 20040072266

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040072266 A1

TITLE: Method of modifying cell motility via compounds and polypeptides that interact with a Wnt pathway

PUBLICATION-DATE: April 15, 2004

INVENTOR-INFORMATION:

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Cohen, Ethan David	Philadelphia	PA	US	

APPL-NO: 10/ 251769

DATE FILED: September 23, 2002

US-CL-CURRENT: 435/7.23, 435/455

ABSTRACT:

The invention provides a novel method for analyzing cell motility by employing the Wnt signaling pathway. Cells and methods are described where Wnt polypeptides have a pronounced and measurable effect on cell motility. Agents that can be used to inhibit or induce cell motility can be screened for and identified.

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application entitled Method of Facilitating Focal Adhesion Kinase-Mediated Cell Motility," filed Sep. 21, 2001, and assigned serial No. _____, (Atty Docket No. 22253-69936) the entire contents of which are hereby incorporated by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (6):

[0007] Integrins are key components of these complexes, as they form the bridge between actin and the extracellular matrix. The cytoskeletal proteins vinculin, talin, and a-actinin are also present in focal complexes, as are several cytoplasmic protein tyrosine kinases, including Src and Focal Adhesion Kinase (FAK) (Critchley, 2000; Liu et al., 2000). The localization of these kinases to focal complexes leads to their activation and the subsequent phosphorylation of their targets. FAK phosphorylates focal adhesion components to induce focal adhesion disassembly. It can also function upstream of P13-kinase, Extracellular signal-regulated kinase (ERK) and Jun-N-terminal kinase (JNK) to regulate diverse cellular processes such as cell proliferation and viability (Parsons et al., 2000).

PGPUB-DOCUMENT-NUMBER: 20040072228

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040072228 A1

TITLE: Card11 NFkB activating polypeptides, nucleic acids, inbred and transgenic animals, and methods of use thereof

PUBLICATION-DATE: April 15, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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APPL-NO: 10/ 632696

DATE FILED: August 1, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60401078 20020802 US

non-provisional-of-provisional 60422614 20021029 US

US-CL-CURRENT: 435/6, 435/194 , 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

The invention provides novel caspase recruitment domain 11 (CARD11), also known as CARMA-1, polypeptides, nucleic acids encoding them and methods for making and using them. In one aspect, the polypeptides of the invention have NFkB activating activity. The invention also provides non-human transgenic animals, e.g., mice, comprising the CARD11 nucleic acids of the invention. The invention also provides pharmaceutical compositions comprising a nucleic acid or polypeptide of the invention. Administration of a pharmaceutical composition of the invention to a subject is used to generate a toleragenic immunological environment in the subject. This can be used to tolerize the subject to an antigen. The invention also provides inbred mouse strains homozygous for a non-wild type CARD11 allele. This genotype results in mice having a phenotype comprising dermatitis, B cell defects and T cell defects.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (4):

[0002] The caspase recruitment domain (CARD) polypeptide can function as a protein-binding module to mediate the assembly of CARD-containing proteins into apoptosis and NF-kappaB signaling complexes. It has been reported that CARD protein 11 (CARD11), also known as CARMA-1, and CARD protein 14 (CARD14) maybe members of a membrane-associated guanylate kinase (MAGUK) family, a class of proteins that functions as molecular scaffolds for the assembly of multiprotein complexes at specialized regions of the plasma membrane. CARD11 and CARD14 have been reported to have homologous structures consisting of an N-terminal CARD domain, a central coiled-coil domain, and a C-terminal tripartite domain

comprised of a PDZ domain, an Src homology 3 domain, and a GUK domain with homology to guanylate kinase. The CARD domains of both CARD11 and CARD14 may associate with the CARD domain of BCL10, a signaling protein that activates NF-kappaB through the I-kappa-B kinase complex in response to upstream stimuli. It was reported that when expressed in cells, CARD11 and CARD14 activate NF-kappa B and induce the phosphorylation of BCL11. These findings suggest that CARD11 and CARD14 are MAGUK family members that function as upstream activators of BCL10 and NF-kappaB signaling. See, e.g., Bertin (2001) J. Biol. Chem. 276(15):11877-11882; WO 01/40468.

PGPUB-DOCUMENT-NUMBER: 20040053931

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040053931 A1

TITLE: Azaindoles

PUBLICATION-DATE: March 18, 2004

INVENTOR-INFORMATION:

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Morley, Andrew D.	Macclesfield	NJ	GB	
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Deprets, Stephanie D.	Morristown	NJ	US	
Edlin, Christopher	Newark	NJ	GB	
Gardner, Charles J.	Royersford	NJ	US	
Kominos, Dorothea	Millington	NJ	US	
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Edwards, Michael	Morristown		US	
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Houille, Olivier	Magstatt-le-bas		FR	
Damour, Dominique	Orly		FR	
Bouchard, Herve	Thiais		FR	
Bezard, Daniel N.A.	Bagnolet		FR	
Carrez, Chantal	Thiais		FR	

APPL-NO: 10/ 177804

DATE FILED: June 21, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60300257 20010622 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
GB	0115109.1	2001GB-0115109.1	June 21, 2001

US-CL-CURRENT: 514/249, 514/300 , 544/350 , 546/113

ABSTRACT:

The invention is directed to physiologically active compounds of general formula (I):-- 1
and compositions containing such compounds; and their prodrugs, and pharmaceutically acceptable salts and solvates of such compounds and their prodrugs, as well as to novel compounds within the scope of formula (I). Such compounds and compositions have valuable pharmaceutical properties, in particular the ability to inhibit kinases.

[0001] This application is entitled to the benefit of earlier filed applications GB 0115109.1, filed Jun. 21, 2001, and U.S. No. 60/300,257 filed Jun. 22, 2001.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (3):

[0004] Biochemical Sciences, 1995, 18, pages 195-197]. The tyrosine kinases include membrane-spanning growth factor receptors such as the epidermal growth factor receptor [S. Iwashita and M. Kobayashi, Cellular Signalling, 1992, 4, pages 123-132], and cytosolic non-receptor kinases such as p56tck, p59fYn, ZAP-70 and csk kinases [C.Chan et. al., Ann. Rev. Immunol., 1994,12, pages 555-592]. Inappropriately high protein kinase activity has been implicated in many diseases resulting from abnormal cellular function. This might arise either directly or indirectly, for example by failure of the proper control mechanisms for the kinase, related for example to mutation, over-expression or inappropriate activation of the enzyme; or by over- or underproduction of cytokines or growth factors also participating in the transduction of signals upstream or downstream of the kinase. In all of these instances, selective inhibition of the action of the kinase might be expected to have a beneficial effect. Spleen tyrosine kinase (Syk) is a 72-kDa cytoplasmic protein tyrosine kinase that is expressed in a variety of hematopoietic cells and is an essential element in several cascades that couple antigen receptors to cellular responses. Thus, Syk plays a pivotal role in signalling of the high affinity IgE receptor, Fc.epsilon.R1, in mast cells and in receptor antigen signalling in T and B lymphocytes. The signal transduction pathways present in mast, T and B cells have common features. The ligand binding domain of the receptor lacks intrinsic tyrosine kinase activity. However, they interact with transducing subunits that contain immunoreceptor tyrosine based activation motifs (ITAMs) [M. Reth, Nature, 1989, 338, pages 383-384]. These motifs are present in both the .beta. and .gamma. subunits of the Fc.epsilon.R1, in the .xi.-subunit the of T cell receptor (TCR) and in the IgG.alpha. and IgG.beta. subunits of the B cell receptor (BCR). [N. S. van Oers and A. Weiss, Seminars in Immunology, 1995, 7, pages 227-236] Upon binding of antigen and multimerization, the ITAM residues are phosphorylated by protein tyrosine kinases of the Src family. Syk belongs to a unique class of tyrosine kinases that have two tandem Src homology 2 (SH2) domains and a C terminal catalytic domain. These SH2 domains bind with high affinity to ITAMs and this SH2-mediated association of Syk with an activated receptor stimulates Syk kinase activity and localises Syk to the plasma membrane.

PGPUB-DOCUMENT-NUMBER: 20040043950

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040043950 A1

TITLE: WT1 antisense oligos for the inhibition of breast cancer

PUBLICATION-DATE: March 4, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Lopez-Berestein, Gabriel	Bellaire	TX	US	
Tari, Ana Maria	Houston	TX	US	
Zapata-Benavides, Pablo	Guadalupe NL		MX	

APPL-NO: 10/ 336253

DATE FILED: January 3, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60345102 20020103 US

US-CL-CURRENT: 514/44

ABSTRACT:

The present invention provides methods for inhibiting the growth of breast cancer cells and methods for treating breast cancers expressing Wilms' Tumor 1 (WT1) gene product using a WT1 antisense oligonucleotide. It further provides methods of predicting breast cancer progression and methods for the screening of candidate substances for activity against breast cancer.

[0001] The present application claims priority to provisional U.S. Patent Application Serial No. 60/345,102 filed Jan. 3, 2002. The entire text of the above referenced applications are incorporated herein by reference and without disclaimer.

---------- KWIC ---------

Detail Description Table CWU - DETL (4):

4TABLE 3 Gene Source Human Disease Function Growth Factors HST/KS Transfection FGF family member INT-2 MMTV promoter FGF family member Insertion INTI/WNTI MMTV promoter Factor-like Insertion SIS Simian sarcoma PDGF B virus Receptor Tyrosine Kinases ERBB/HER Avian Amplified, deleted EGF/TGF-.alpha./ erythroblastosis squamous cell Amphiregulin/ virus; ALV cancer; Hetacellulin promoter glioblastoma receptor insertion; amplified human tumors ERBB-2/NEU/HER- Transfected from rat Amplified breast, Regulated by NDF/ 2 Glioblastomas ovarian, gastric Heregulin and cancers EGF-Related factors FMS SM feline sarcoma CSF-1 receptor virus KIT HZ feline sarcoma MGF/Steel receptor virus Hematopoieis TRK Transfection from NGF (nerve growth human colon Factor) receptor cancer MET Transfection from Scatter factor/HGF human Receptor osteosarcoma RET Translocations and Sporadic

thyroid Orphan receptor Tyr point mutations cancer; Kinase familial medullary thyroid cancer; multiple endocrine neoplasias 2A and 2B ROS URII avian sarcoma Orphan receptor Tyr Virus Kinase PDGF receptor Translocation Chronic TEL(ETS-like Myelomonocytic transcription Leukemia factor)/ PDGF receptor gene Fusion TGF-.beta. receptor Colon carcinoma mismatch mutation target NONRECEPTOR TYROSINE KINASES ABI. Abelson Mul. V Chronic Interact with RB, myelogenous RNA leukemia polymerase, CRK, translocation CBL with BCR FPS/FES Avian Fujinami SV; GA FeSV LCK Mul. V (murine Src family; T-cell leukemia signaling; interacts virus) promoter CD4/CD8 T-cells insertion SRC Avian Rous Membrane- sarcoma associated Tyr Virus kinase with signaling function; activated by receptor kinases YES Avian Y73 virus Src family; signaling SER/THR PROTEIN KINASES AKT AKT8 murine Regulated by retrovirus PJ(3)K?; regulate 70-kd S6 k? MOS Moloney murine SV GVBD; cystostatic factor; MAP kinase kinase PIM-1 Promoter insertion Mouse RAF/MIL 3611 murine SV; Signaling in RAS MH2 Pathway avian SV MISCELLANEOUS CELL SURFACE APC Tumor suppressor Colon cancer Interacts with catenins DCC Tumor suppressor Colon cancer CAM domains E-cadherin Candidate tumor Breast cancer Extracellular Suppressor homotypic binding; intracellular interacts with catenins PTC/NBCCS Tumor suppressor Nevoid basal cell 12 transmembrane and cancer domain; signals Drosophilia syndrome (Gorline through Gli homology syndrome) homogue CI to antagonize hedgehog pathway TAN-1 Notch Translocation T-ALI. Signaling? homologue MISCELLANEOUS SIGNALING BCL-2 Translocation B-cell lymphoma Apoptosis CBL Mu Cas NS-1 V Tyrosine-Phosphorylated RING finger interact Abl CRK CT1010 ASV Adapted SH2/SH3 interact Abl DPC4 Tumor suppressor Pancreatic cancer TGF-.beta.-related signaling Pathway MAS Transfection and Possible angiotensin Tumorigenicity Receptor NCK Adaptor SH2/SH3 GUANINE NUCLEOTIDE EXCHANGERS AND BINDING PROTEINS BCR Translocated with Exchanger; protein ABL Kinase in CML DBL Transfection Exchanger GSP NF-1 Hereditary tumor Tumor suppressor RAS GAP Suppressor neurofibromatosis OST Transfection Exchanger Harvey-Kirsten, N-HaRat SV; Ki Point mutations in Signal cascade RAS RaSV; many Balb-MoMuSV; human tumors Transfection VAV Transfection S112/S113; exchanger NUCLEAR PROTEINS AND TRANSCRIPTION FACTORS BRCA1 Heritable suppressor Mammary Localization cancer/ovarian unsettled cancer BRCA2 Heritable suppressor Mammary cancer Function unknown ERBA Avian thyroid hormone erythroblastosis receptor Virus (transcription) ETS Avian E26 virus DNA binding EVII MuLV promotor AML Transcription factor Insertion FOS FBI/FBR murine 1 transcription osteosarcoma factor viruses with c-JUN GLI Amplified glioma Glioma Zinc finger; cubitus interrupts homologue is in hedgehog signaling pathway; inhibitory link PTC and hedgehog HMGI/LIM Translocation Lipoma Gene fusions high t(3:12) mobility group t(12:15) HMGI-C (XT hook) and transcription factor LIM or acidic domain JUN ASV-17 Transcription factor AP-1 with FOS MLL/VHRX + Translocation/fusion Acute myeloid Gene fusion of ELI/MEN ELL with MLL leukemia DNA- Trithorax-like gene binding and methyl transferase MLL with ELI RNA pol II elongation factor MYB Avian DNA binding myeloblastosis Virus MYC Avian MC29; Burkitt's lymphoma DNA binding with Translocation B- MAX partner; cell cyclin Lymphomas; regulation; interact promoter RB?; regulate Insertion avian apoptosis? leukosis Virus N-MYC Amplified Neuroblastoma L-MYC Lung cancer REL Avian NF-.kappa.B family transcription factor Retriculoendotheliosis Virus SKI Avian SKV770 Transcription factor Retrovirus VHL Heritable suppressor Von Hippel-Landau Negative regulator syndrome or elongin; transcriptional elongation complex WT-1 Wilms' tumor Transcription factor CELL CYCLE/DNA DAMAGE RESPONSE ATM Hereditary disorder Ataxia- Protein/lipid kinase telangiectasia homology; DNA damage response upstream in P53 pathway BCL-2 Translocation Follicular Apoptosis lymphoma FACC Point mutation Fanconi's anemia group

PGPUB-DOCUMENT-NUMBER: 20040038207

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040038207 A1

TITLE: Gene expression in bladder tumors

PUBLICATION-DATE: February 26, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Orntoft, Torben F.	Aabyhoj		DK	

APPL-NO: 09/ 951968

DATE FILED: September 14, 2001

RELATED-US-APPL-DATA:

child 09951968 A1 20010914

parent division-of 09510643 20000222 US UNKNOWN

US-CL-CURRENT: 435/6

ABSTRACT:

Methods for analyzing tumor cells, particularly bladder tumor cells employ gene expression analysis of samples. Gene expression patterns are formed and compared to reference patterns. Alternatively gene expression patterns are manipulated to exclude genes which are expressed in contaminating cell populations. Another alternative employs subtraction of the expression of genes which are expressed in contaminating cell types. These methods provide improved accuracy as well as alternative basis for analysis from diagnostic and prognostic tools currently available.

[0001] This application claims the benefit of U.S. Provisional Application No. 60/121,124, filed Feb. 22, 1999, which is hereby incorporated by reference in its entirety.

---------- KWIC ---------

Detail Description Table CWU - DETL (102):
X17059_s_at Human NAT1 gene for arylamine 20 29 20 24 54 20 N-acetyltransferase X17093_at Human HLA-F gene for human leukocyte 435 2844 1104 565 338 963 antigen F X17094_at Human fur mRNA for funn 20 20 41 52 31 20 X17098_at Human PSG10 mRNA for pregnancy specific 20 20 20 20 74 20 glycoprotein 10 X17206_at Human mRNA for LLRep3 4928 4634 6071 5895 2199 4017 X17254_at Human mRNA for the transcription factor Eryf1 20 20 20 24 20 20 X17380_ma1_at Human HOX 5 1 gene for HOX 5 1 protein 20 20 20 20 20 20 X17567_s_at H. sapiens RNA for snRNP protein B 206 261 636 558 467 391 X17576_at Human melanoma mRNA for nck "protein," showing 49 20 25 20 20 26 homology to src X17620_at Human mRNA tor Nm23 "protein," involved in 139 178 195 200 248 269 developmental regulation (homolog to Drosophila Awd protein)

X17622_at Human HBK2 mRNA for potassium channel protein 20 20 37 27 90 20 X17644_s_at Human GST1-Hs mRNA for GTP-binding protein 22 20 20 91 20 27 X17648_at Human mRNA for granulocyte-macrophage 33 25 36 20 98 67 colony-stimulating factor receptor (hGM-CSF-R) X17651_at Human Myf-4 mRNA for myogenic determination 61 35 54 55 128 113 factor X51345_at Human jun-B mRNA for JUN-B protein 1446 1817 1394 1497 502 609 X51362_s_at Human mRNA for dopamine D2 receptor 40 123 186 115 2181 103 X51405_at Human mRNA for carboxypeptidase E 28 27 20 20 20 20 (EC 3.4.17.10) X51408_at Human mRNA for n-chimaerin 24 32 37 20 26 80 X51417_at Human mRNA for steroid hormone receptor hERR2 20 20 20 20 311 20 X51420_at Human mRNA for tyrosinase-related protein 20 20 20 20 20 20 X51435_s_at Human PRDII-BF1 gene for a DMA-binding protein 30 53 73 26 32 22 X51441_at 31 71 34 25 237 20 X51441_s_at Human mRNA for serum amyloid A (SAA) protein 20 50 20 20 20 20 "partial," clone pAS3-alpha X51466_at Human mRNA for elongation factor 2 1116 758 1732 1754 354 608 X51521_at Human mRNA for ezrin 376 485 621 425 337 501 X51602_at Human fit mRNA for receptor-related tyroslne 20 20 20 20 20 20 kinase X51630_at Human Wilms tumor WT1 mRNA for zinc finger 20 20 22 20 20 20 "protein," Krueppel-like X51688_at Human mRNA for cyclin A 30 20 20 20 30 20 X51698_s_at H. sapiens spasmolvtic polyypeptide (SP) mRNA 20 20 87 20 20 20 X51730_at Human mRNA and promoter DNA for progesterone 20 20 20 20 59 33 receptor X51755_cds5_s_at Ig "light-chain," partial Ke-Oz-polypeptide; 20 20 20 20 116 20 Author-given protein sequence is in conflict with the conceptual translation gene extracted from X51757_at Hurrian heat-shock protein HSP70B' gene 20 20 20 20 20 26 X51801_at Human OP-1 mRNA for osteogenic protein 20 68 37 52 49 20 X51804_at Human PMI gene for a putative receptor protein 42 20 32 24 20 66 X51823_at 20 20 20 20 20 20 X516233s_at Human mRNA for B-subunit of coagulation factor XIII 20 20 20 20 20 20 (FXIIIB) (partial) /gb = X51823 /ntype = RNA X51952_xpt1_at Human UCP gene for uncoupling protein exons 1 and 2 139 20 20 20 20 20 X51953_at Human UCP gene for uncoupling protein exons 3 and 20 20 20 20 20 20 4 /gb = X51953 /ntype = DNA /annot = exon X51954_at Human UCP gene for uncoupling protein exon 5 /gb = 20 20 24 20 20 20 X51954 /ntype = DNA /annot = exon X51956_ma1_at Human ENO2 gene for neuron specific (gamma) enolase 20 23 54 66 50 39 X51985_at Human LAG-3 mRNA for Correlated protein involved in 20 20 20 20 20 20 lymphocyte activation X52001_at H. sapiens endothelin 3 mRNA 20 20 20 20 20 38 X52003_at H. sapiens pS2 protein gene 321 63 1845 71 20 46 X52005_at H. sapiens skeletal embryonic myosin light chain 1 20 20 20 20 34 20 (MLC1) mRNA X52008_at H. sapiens alpha-2 strychnine binding subunit of 41 20 30 34 20 20 inhibitory glycine receptor mRNA X52009_s_at 20 20 20 20 88 39 X52011_at H. sapiens MYF6 gene encoding a muscle 24 23 20 20 86 20 determination factor X52022_at H. sapiens RNA for type VI collagen alpha3 chain 231 138 20 20 20 20 X52056_at Human mRNA for api-1 proto-oncogene 20 20 20 20 20 20 X52075_ma1_at Human gene for sialophorin (CD43) 20 20 20 20 44 20 X52142_at Human mRNA for CTP synthetase (EC 6.3 4 2) 20 20 20 23 20 33 X52151_at Homo sapiens arylsulphatase A "mRNA," 96 27 90 84 117 135 complete cds X52192_at H. sapiens RNA for c-fes 101 104 164 115 219 210 X52213_s_at H. sapiens itk mRNA 20 20 20 20 20 20 X52221_at H. sapiens ERCC2 "gene," exons 1 & 2 (partial) 20 20 20 30 37 62 X52228_at Human mRNA for secreted epithelial tumour 20 20 20 20 20 20 mucin antigen X52282_s_at Human mRNA for_atrial natriuretic peptide 20 20 20 20 20 20 clearance receptor (ANP-C receptor) X52425_at Human IL-4-R mRNA for the interteukin 4 91 73 124 103 255 179 receptor X52426_s_at H. sapiens mRNA for cytokeratin 13 13355 5075 5448 3230 340 850 X52429_at Human PKC alpha mRNA for protein kinase 48 31 59 39 20 52 C alpha X525 0_at Human mRNA for tyrosine aminotransferase 41 20 31 20 29 20 (TAf)fEC 2.6 1.5) X52541_at Human mRNA for early growth response 160 39 20 20 20 50 protein 1 (hEGRI) X52599_at Human mRNA for beta nerve growth factor 20 20 20 20 20 20 X52611_s_at Human mRNA for transcription factor AP-2 20 25 20 20 20 41 X52638_at Human mRNA for "6-phosphofructo-2-kinase/ 26 51 65 49 20 20 fructose-2,6-bisphosphatase" (EC "2.7 1 105," EC 3 1 3.46) X52730_ma1_at

Human gene for phenylethanolamine N-methylase 222 192 242 196 157 257 (PNMT) (EC 2 1 1 28) X52773_at Human mRNA for retinoic acid receptor-like 20 20 20 20 20 20 protein X52851_ma1_at Human cyclophilm gene for cyclophilin 1727 1384 1492 1802 1382 1695 (EC 5 2 1 8) X52882_at Human t-complex poiypeptide 1 gene 127 66 204 258 68 241 X52889_at Human gene for cardiac beta myosin heavy chain 20 20 132 24 678 217 X52896_s_at H. sapiens RNA for dermal fibroblast elastin 21 20 20 20 263 142 X52943_at Human mRNA for ATF-a transcription factor 43 73 71 57 136 89 X52947_at Human mRNA for cardiac gap junction protein 188 39 50 79 113 31 X52966_at Human mRNA for ribosomal protein L35a 1361 1485 1662 1388 359 1319 X52979_ma1_s_at SmB protein gene extracted from Human gene 209 218 528 408 379 410 for small nuclear ribonucleoproteins SmB and SmB' X53002_s_at Human mRNA for integrm beta-5 subunit 59 20 102 52 56 59 X53065_f_at Human SPR2-1 gene for small proline rich 20 20 55 59 145 109 protein (exon 2) X53296_s_at H. sapiens mRNA for IRAP 1652 217 219 237 20 144 X53331_at Human mRNA for matrix Gla protein 1079 293 82 72 375 179 X53390_s_at Human mRNA for upstream binding factor 20 143 167 140 2651 144 (hUBF) X53414_at Human mRNA for peroxisomai L-alanine: 221 100 52 38 751 20 glyoxylate aminotransferase X53416_at Human mRNA for actin-binding protein (filamin) 158 90 55 33 20 60 (ABP-280) X53586_ma1_at Human mRNA for integrin alpha 6. 57 43 153 97 20 28 X53587_at Human mRNA for integrin beta 4 20 30 274 329 40 20 X53595_s_at Human mRNA for beta-2-glycoprotein I 20 73 75 20 20 34 (apolipoprotein H) X53683_at Human LAG-1 mRNA 20 20 20 20 31 48 X53742_at H. sapiens mRNA fof fibulin-1 B 20 20 20 20 20 20 X53777_at Human L23 mRNA for putative ribosomal protein 1915 2851 2260 2066 302 1098 X53793_at H. sapiens ADE2H1 mRNA" showing homologies to 39 34 61 41 64 75 SAICAR synthetase and AIR carboxylase of the purine pathway (EC "6 3 2 6," EC 4 1 X53795_at Human R2 mRNA for an inducible membrane 20 85 21 24 96 20 protein X53800_s_at Human mRNA for macrophage inflammatory 20 20 20 20 20 21 protein-2beta (MIP2beta) X53961_at Human mRNA for lactoferrin 20 20 20 20 20 20 20 X54131_at Human HPTP beta mRNA for protein tyrosine 20 20 20 20 20 20 phosphatase beta X54150_at Human mRNA for Fc receptor 20 20 20 20 20 20 X54162_at Human mRNA for a 64 Kd autoantigen expressed 47 24 20 20 20 20 in thyroid and extra-ocular muscle X54199_s_at Human mRNA for GARS-AIRS-GART 20 20 20 20 50 20 X54232_at Human mRNA for heparan sulfate proteaglycan 131 118 245 235 20 20 (glypican) X54304_at Human mRNA for myosin regulatory light chain 350 187 315 301 132 357 X54326_at H. sapiens mRNA for glutamlnyl-tRNA syrrthetase 39 20 31 35 33 20 X54380_at Human mRNA (or pregnancy zone protein 20 20 20 20 20 20 X54457_s_at 20 20 20 20 20 20 X54489_ma1_at Human gene (or melanoma growth stimulatory 43 85 20 20 20 23 activity (MGSA) X54637_at Human tyk2 mRNA for non-receptor protein 20 20 53 55 98 91 tyrosIne kinase X54667_at 27 29 204 75 20 20 X54667_s_at H. sapiens mRNA for cystatin S 110 129 299 153 320 135 X54673_at H. sapiens GAT1 mRNA for GABA transporter 20 20 20 20 20 20 X54741_at Human CYPXIB2 gene for_atdosterone synthase 20 20 20 20 20 20 X54816_at Human gene for "alpha-1-microglobulin-bikunin," 20

PGPUB-DOCUMENT-NUMBER: 20040028661

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040028661 A1

TITLE: Expansion of cells using thrombopoietin and anti-transforming growth factor-beta

PUBLICATION-DATE: February 12, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bartelmez, Stephen H.	Seattle	WA	US	

APPL-NO: 10/ 213957

DATE FILED: August 7, 2002

US-CL-CURRENT: 424/93.21, 435/366 , 514/44

ABSTRACT:

The invention features a method for the expansion of hematopoietic stem cells using a combination of a thrombopoietin agonist and a transforming growth factor-beta blocking agent in the absence of stem cell factor. The invention also features a hematopoictic stem cell composition that has been expanded using a combination of a thrombopoietin agonist and a transforming growth factor-beta blocking agent in the absence of stem cell factor, as well as methods of using an expanded hematopoietic stem cell composition to restore or supplement an immune system and/or blood forming system compromised by, for example, radiation or chemotherapy.

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Detail Description Paragraph - DETX (21):

[0053] By way of example, TPO agonists that activate the mpl receptor signal transduction pathway would activate a JAK, for example, JAK1, JAK2, JAK3 and TYK2, which are activated upon ligand binding to the mpl receptor. Activation of JAKs results in phosphorylation of multiple cellular proteins, including the associated cytokine receptors and the JAKs themselves (reviewed in Ihle, Adv. Immunol., 60:1-35, 1995). The phosphorylated tyrosines are potential docking sites for proteins containing specific phosphotyrosine binding domains (e.g. Src homology (SH)2 and phosphotyrosine binding (PTB) domains). Specific signaling proteins are thereby recruited into the cytokine signaling networks. Since JAK2 physically associates with its activating receptors (via the proline-rich region) and is activated within seconds after receptor engagement, it appears that JAK2 activation is an early, perhaps initiating step in signal transduction by mpl receptor-ligand interaction. A number of signaling molecules that appear to be activated by recruitment to JAK2-mpl-receptor complexes include: 1) Shc proteins, which lie upstream of Ras and the mitogen-activated protein (MAP) kinases ERKs 1 and 2, which are implicated in the regulation of cellular growth and/or differentiation. 2) the insulin receptor substrates (IRS) 1 and 2; and 3) the signal transducers and activators of transcription (STAT) 1, 3, 5a, and 5b, which have been implicated as regulators of transcription of a variety of genes. Agonists of the mpl receptor signaling pathway that are able to activate the JAK/STAT pathway at

the level of protein-protein interaction or protein-DNA interaction are also included in the methods of the invention.

PGPUB-DOCUMENT-NUMBER: 20040018513

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040018513 A1

TITLE: Classification and prognosis prediction of acute lymphoblastic leukemia by gene expression profiling

PUBLICATION-DATE: January 29, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Downing, James R.	Cordova	TN	US	
Yeoh, Eng-Juh	Singapore	MS	SG	
Wilkins, Dawn E.	Oxford		US	
Wong, Limsoon	Singapore		SG	

APPL-NO: 10/ 391271

DATE FILED: March 18, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60367144 20020322 US

US-CL-CURRENT: 435/6

ABSTRACT:

The present invention provides methods and compositions useful for diagnosing and choosing treatment for leukemia patients. The claimed methods include methods of assigning a subject affected by leukemia to a leukemia risk group, methods of predicting whether a subject affected by leukemia has an increased risk of relapse, methods of predicting whether a subject affected by leukemia has an increased risk of developing secondary acute myeloid leukemia, methods to aid in the determination of a prognosis for a subject affected by leukemia, methods of choosing a therapy for a subject affected by leukemia, and methods of monitoring the disease state in a subject undergoing one or more therapies for leukemia. The claimed compositions include arrays having capture probes for the differentially-expressed genes of the invention, computer readable media having digitally-encoded expression profiles associated with leukemia risk groups, and kits for diagnosing and choosing therapy for leukemia patients.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 60/367,144 filed Mar. 22, 2002, which is hereby incorporated in its entirety by reference herein.

---------- KWIC ---------

Detail Description Table CWU - DETL (14):
13TABLE 11 Genes selected by CFS for: Hyperdiploid >50 Above/ Affymetrix Reference Below number Gene Name GeneSymbol number Mean 1 36620_at superoxide dismutase 1 soluble SOD1 X02317 Above amyotrophic lateral

sclerosis 1 adult 2 37350_at clone 889N15 on chromosome PSMD10 AL031177 Above Xq22.1-22.3. Contains part of the gene for a novel protein similar to X. laevis Cortical Thymocyte Marker CTX 3 41724_at accessory proteins BAP31/BAP29 DXS1357E X81109 Above 4 38738_at SMT3 suppressor of mif two 3 yeast SMT3H1 X99584 Above homolog 1 5 40480_s_at FYN oncogene related to SRC FGR FYN M14333 Above YES 6 38518_at sex comb on midleg Drosophila like 2 SCML2 Y18004 Above 7 31492_at muscle specific gene M9 AB019392 Below 8 35688_g_at mature T-cell proliferation 1 MTCP1 Z24459 Above 9 35939_s_at POU domain class 4 transcription POU4F1 L20433 Above factor 1 10 36128_at transmembrane trafficking protein TMP21 L40397 Above 11 37014_at myxovirus influenza resistance 1 MX1 M33882 Above homolog of murine interferon-inducible protein p78 12 34374_g_at upstream regulatory element binding UREB1 Z97054 Above protein 1 13 688_at proteasome prosome macropain 26S PSMC1 L02426 Above subunit ATPase 1 14 39878_at protocadherin 9 PCDH9 AI524125 Below 15 38771_at histone deacetylase 1 HDAC1 D50405 Below 16 865_at ribosomal protein S6 kinase 90 kD RPS6KA3 U08316 Above polypeptide 3 17 41143_at calmodulin (CALM1) gene CALM1 U12022 Above 18 39867_at Tu translation elongation factor TUFM S75463 Below mitochondrial 19 41470_at prominin mouse like 1 PROML1 AF027208 Above 20 41503_at KIAA0854 protein KIAA0854 AB020661 Below 21 2039_s_at FYN oncogene related to SRC FGR FYN M14333 Above YES 22 36845_at KIAA0136 protein KIAA0136 D50926 Above 23 36940_at TGFB1-induced anti-apoptotic factor 1 TIAF1 D86970 Above 24 32236_at ubiquitin-conjugating enzyme E2G 2 UBE2G2 AF032456 Above homologous to yeast UBC7 25 36885_at spleen tyrosine kinase SYK L28824 Below 26 40200_at heat shock transcription factor 1 HSF1 M64673 Below 27 40842_at U1 snRNP-specific protein A gene SNRPA M60784 Below 28 40514_at hypothetical 43.2 kD protein LOC51614 AF091085 Below 29 41222_at signal transducer and activator of STAT6 AF067575 Below transcription 6 (STAT6) gene 30 1294_at ubiquitin-activating enzyme E1-like UBE1L L13852 Below 31 34315_at AFG3 ATPase family gene 3 yeast AFG3L2 Y18314 Above like 2 32 39806_at DKFZP547E2110 protein DKFZP547E2110 AL050261 Above 33 40875_s_at small nuclear ribonucleoprotein 70 kD SNRP70 X06815 Below polypeptide RNP antigen 34 38458_at cytochrome b5 (CYB5) gene CYB5 L39945 Above 35 1817_at prefoldin 5 PFDN5 D89667 Below 36 34709_r_at stromal antigen 2 STAG2 Z75331 Above 37 33447_at myosin light polypeptide regulatory MLCB X54304 Above non-sarcomeric 20 kD 38 1077_at recombination activating gene 1 RAG1 M29474 Below 39 1915_s_at v-fos FBJ murine osteosarcoma viral FOS V01512 Above oncogene homolog 40 38854_at KIAA0635 gene product KIAA0635 AB014535 Above 41 37732_at RING1 and YY1 binding protein RYBP AL049940 Above 42 35940_at POU domain class 4 transcription POU4F1 X64624 Above factor 1 43 34733_at splicing factor 3a subunit 1 120 kD SF3A1 X85237 Below 44 245_at selectin L lymphocyte adhesion SELL M25280 Below molecule 1 45 40146_at RAP1B member of RAS oncogene RAP1B AL080212 Below family 46 40104_at serine/threonine kinase 25 Ste20 yeast STK25 D63780 Below homolog 47 430_at nucleoside phosphorylase NP X00737 Above 48 36899_at special AT-rich sequence binding SATB1 M97287 Below protein 1 binds to nuclear matrix/scaffold-associating DNA s 49 35727_at hypothetical protein FLJ20517 FLJ20517 AI249721 Below 50 38649_at KIAA0970 protein KIAA0970 AB023187 Below 51 36107_at ATP synthase H transporting ATP5J AA845575 Above mitochondrial F0 complex subunit F6 52 38789_at transketolase Wernicke-Korsakoff TKT L12711 Below syndrome 53 39301_at calpain 3 p94 CAPN3 X85030 Below 54 41278_at BAF53 BAF53A AF041474 Below 55 41162_at protein phosphatase 1G formerly 2C PPM1G Y13936 Below magnesium-dependent gamma isoform 56 37819_at hypothetical protein LOC54104 AF007130 Below 57 38717_at DKFZP586A0522 protein DKFZP586A0522 AL050159 Below 58 40019_at ecotropic viral integration site 2B EVI2B M60830 Above 59 39489_g_at protocadherin 9 PCDH9 W27720 Below 60 857_at protein phosphatase 1A formerly 2C PPM1A S87759 Above magnesium-dependent alpha isoform 61 32804_at RNA binding motif protein 5 RBM5 AF091263 Below 62 37676_at phosphodiesterase 8A PDE8A AF056490 Below 63 1519_at v-ets avian erythroblastosis virus E26 ETS2 J04102 Above oncogene

homolog 2 64 37680_at A kinase PRKA anchor protein gravin AKAP12 U81607 Below 12 65 548_s_at spleen tyrosine kinase SYK S80267 Below 66 39797_at KIAA0349 protein KIAA0349 AB002347 Above 67 32789_at nuclear cap binding protein subunit 2 NCBP2 AA149428 Below 20 kD 68 38091_at lectin galactoside-binding soluble 9 LGALS9 Z49107 Below galectin 9 69 41223_at cytochrome c oxidase subunit Va COX5A M22760 Below 70 933_f_at zinc finger protein 91 HPF7 HTF10 ZNF91 L11672 Below 71 37012_at capping protein actin filament muscle CAPZB U03271 Below Z-line beta 72 35214_at UDP-glucose dehydrogenase UGDH AF061016 Above 73 32434_at myristoylated alanine-rich protein MACS D10522 Above kinase C substrate MARCKS 80K-L 74 38345_at centrosomal protein 1 CEP1 AF083322 Below 75 40404_s_at CDC16 cell division cycle 16 S. CDC16 U18291 Below cerevisiae homolog 76 39096_at SON DNA binding protein SON AB028942 Above 77 33429_at DKFZP586M1523 protein DKFZP586M1523 AL050225 Above 78 40641_at TBP-associated factor 172 TAF-172 AF038362 Above 79 41381_at KIAA0308 protein KIAA0308 AB002306 Below 80 35135_at Homo sapiens Similar to CG15084 X13956 Below gene product clone MGC 10471 mRNA complete cds 81 39421_at runt-related transcription factor 1 RUNX1 D43969 Below acute myeloid leukemia 1 aml1 oncogene 82 195_s_at caspase 4 apoptosis-related cysteine CASP4 U28014 Below protease 83 36898_r_at primase polypeptide 2A 58 kD PRIM2A X74331 Above 84 38792_at spermine synthase SMS AD001528 Above 85 32643_at glucan 1 4-alpha-branching enzyme 1 GBE1 L07956 Below glycogen branching enzyme Andersen disease glycogen storage disease type IV 86 38808_at cell membrane glycoprotein 110000M GP110 D64154 Below r surface antigen 87 36062_at Leupaxin LPXN AF062075 Below 88 300_f_at transcription factor BTF3 homolog HG4518- Below (GB: M90355) HT4921 89 1979_s_at nucleolar protein 1 120 kD NOL1 X55504 Below 90 32230_at eukaryotic translation initiation factor EIF3S2 U39067 Below 3 subunit 2 beta 36 kD 91 39893_at guanine nucleotide binding protein G GNG7 AB010414 Below protein gamma 7 92 34651_at catechol-O-methyltransferase COMT M58525 Above 93 1052_s_at CCAAT/enhancer binding protein CEBPD M83667 Below C/EBP delta 94 36272_r_at peripheral myelin protein 2 PMP2 X62167 Below 95 2044_s_at retinoblastoma 1 including RB1 M15400 Below osteosarcoma 96 32135_at sterol regulatory element binding SREBF1 U00968 Below transcription factor 1

PGPUB-DOCUMENT-NUMBER: 20040013691

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040013691 A1

TITLE: Immunotoxin as a therapeutic agent and uses thereof

PUBLICATION-DATE: January 22, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Rosenblum, Michael G.	Sugarland	TX	US	

APPL-NO: 10/ 460774

DATE FILED: June 12, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60388133 20020612 US

US-CL-CURRENT: 424/234.1, 424/185.1 , 435/4 , 435/6

ABSTRACT:

The present invention further provides insight into the mechanism of action of immunotoxins in disease states such as hyperproliferative disease states. The present invention provides a novel method of treating diseases using immunotoxins and gene expression profiling to identify genes that are modulated by immunotoxin therapy.

---------- KWIC ---------

Summary of Invention - Table CWU - BSTL (1):

1TABLE 1 Gene Source Human Disease Function GROWTH FACTORS HST/KS Transfection FGF family member INT-2 MMTV promoter FGF family member Insertion INTI/ MMTV promoter Factor-like WNTI Insertion SIS Simian sarcoma virus PDGF B RECEPTOR TYROSINE KINASES ERBB/ Avian erythroblastosis Amplified, de- EGF/TGF-/ HER virus; ALV promoter leted squamous Amphiregulin/ insertion; amplified cell cancer; Hetacellulin human tumors glioblastoma receptor ERBB-2/ Transfected from rat Amplified Regulated by NDF/ NEU/ Glioblastomas breast, ovarian, Heregulin and EGF- HER-2 gastric cancers Related factors FMS SM feline sarcoma CSF-1 receptor virus KIT HZ feline sarcoma MGF/Steel receptor virus Hematopoieis TRK Transfection from NGF (nerve growth human colon cancer Factor) receptor MET Transfection from Scatter factor/HGF human osteosarcoma Receptor RET Translocations and Sporadic thy- Orphan receptor Tyr point mutations roid cancer; Kinase familial medul- lary thyroid cancer; multi- ple endocrine neoplasias 2A and 2B ROS URII avian sarcoma Orphan receptor Tyr Virus Kinase PDGF Translocation Chronic TEL(ETS-like receptor Myelomono- transcription factor)/ cytic Leukemia PDGF receptor gene Fusion TGF- Colon receptor carcinoma mis- match mutation target NONRECEPTOR TYROSINE KINASES ABL Abelson Mul. V Chronic Interact with RB, myelogenous RNA polymerase, leukemia trans- CRK, CBL location with BCR FPS/FES Avian Fujinami SV; GA FeSV LCK Mul. V (murine Src family; T cell leukemia virus) pro- signaling; interacts moter insertion CD4/CD8 T cells SRC Avian Rous sarcoma Membrane-associa- Virus ted

Tyr kinase with signaling function; activated by receptor kinases YES Avian Y73 virus Src family; signaling SER/THR PROTEIN KINASES AKT AKT8 murine Regulated by retrovirus PI(3)K; regulate 70-kd S6 k MOS Maloney murine SV GVBD; cystostatic factor; MAP kinase kinase PIM-1 Promoter insertion Mouse RAF/MIL 3611 murine SV; MH2 Signaling in RAS avian SV Pathway MISCELLANEOUS CELL SURFACE APC Tumor suppressor Colon cancer Interacts with catenins DCC Tumor suppressor Colon cancer CAM domains E-cadherin Candidate tumor Breast cancer Extracellular homo- Suppressor typic binding; intra- cellular interacts with catenins PTC/ Tumor suppressor and Nevoid basal 12 transmembrane NBCCS Drosophilia homology cell cancer domain; signals syndrome through Gli (Gorline homogue CI to syndrome) antagonize hedge- hog pathway TAN-1 Translocation T-ALL Signaling Notch homo- logue MISCELLANEOUS SIGNALING BCL-2 Translocation B-cell Apoptosis lymphoma CBL Mu Cas NS-1 V Tyrosine- Phosphorylated RING finger interact Abl CRK CT1010 ASV Adapted SH2/SH3 interact Abl DPC4 Tumor suppressor Pancreatic TGF--related cancer signaling Pathway MAS Transfection and Possible angiotensin Tumorigenicity Receptor NCK Adaptor SH2/SH3 GUANINE NUCLEOTIDE EXCHANGERS AND BINDING PROTEINS BCR Translocated Exchanger; protein with ABL in Kinase CML DBL Transfection Exchanger GSP NF-1 Hereditary tumor Tumor sup- RAS GAP Suppressor pressor neuro- fibromatosis OST Transfection Exchanger Harvey- HaRat SV; Ki RaSV; Point mutations Signal cascade Kirsten, Balb-MoMuSV; in many human N-RAS Transfection tumors VAV Transfection S112/S113; exchanger NUCLEAR PROTEINS AND TRANSCRIPTION FACTORS BRCA1 Heritable suppressor Mammary Localization cancer/ovarian unsettled cancer BRCA2 Heritable suppressor Mammary Function unknown cancer ERBA Avian erythroblastosis thyroid hormone Virus receptor (transcription) ETS Avian E26 virus DNA binding EVII MuLV promotor AML Transcription factor Insertion FOS FBI/FBR murine transcription factor osteosarcoma viruses with c-JUN GLI Amplified glioma Glioma Zinc finger; cubitus interruptus homo- logue is in hedgehog signaling pathway; inhibitory link PTC and hedgehog HMGI/ Translocation t(3:12) Lipoma Gene fusions high LIM t(12:15) mobility group HMGI-C (XT-hook) and transcription factor LIM or acidic domain JUN ASV-17 Transcription factor AP-1 with FOS MLL/ Translocation/fusion Acute myeloid Gene fusion of VHRX + ELL with MLL leukemia DNA-binding and ELI/MEN Trithorax-like gene methyl transferase MLL with ELI RNA pol II elongation factor MYB Avian myeloblastosis DNA binding Virus MYC Avian MC29; Burkitt`s DNA binding with Translocation B-cell lymphoma MAX partner; Lymphomas; promoter cyclin regulation; Insertion avian interact RB; regulate leukosis Virus apoptosis N-MYC Amplified Neuroblastoma L-MYC Lung cancer REL Avian NF-B family Retriculoendotheliosis transcription factor Virus SKI Avian SKV770 Transcription factor Retrovirus VHL Heritable suppressor Von Hippel- Negative regulator Landau or elongin; trans- syndrome criptional elongation complex WT-1 Wilm's tumor Transcription factor CELL CYCLE/DNA DAMAGE RESPONSE ATM Hereditary disorder Ataxia- Protein/lipid kinase telangiectasia homology; DNA damage response upstream in P53 pathway BCL-2 Translocation Follicular Apoptosis lymphoma FACC Point mutation Fanconi's anemia group C (predispo- sition leukemia MDA-7 Fragile site 3p14.2 Lung Histidine triad-re- carcinoma lated diadenosine 5,3-tetraphosphate asymmetric hydrolase hML1/ HNPCC Mismatch repair; MutL MutL Homologue hMSH2/ HNPCC Mismatch repair; MutS MutS Homologue

PGPUB-DOCUMENT-NUMBER: 20040009939

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040009939 A1

TITLE: Methods of enhancing immune induction involving MDA-7

PUBLICATION-DATE: January 15, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Chada, Sunil	Missouri City	TX	US	
Pataer, Abujiang	Houston	TX	US	
Mhashilkar, Abner	Houston	TX	US	
Ramesh, Rajagopal	Sugarland	TX	US	
Roth, Jack	Houston	TX	US	
Swisher, Steve	Fresno	TX	US	

APPL-NO: 10/ 378590

DATE FILED: March 3, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60404932 20020821 US

non-provisional-of-provisional 60370335 20020405 US

non-provisional-of-provisional 60361755 20020305 US

US-CL-CURRENT: 514/44, 424/93.2 , 514/12

ABSTRACT:

The present invention relates to compositions and methods for the enhancing or inducing an immune response against an immunogenic molecule by indirectly activating PKR. More specifically, immunotherapy is improved by co-administering a MDA-7 polypeptide with an immunogenic molecule against which an immune response is desired. Such immunotherapies include cancer vaccines, and compositions thereof are described.

[0001] This application claims the priority of U.S. Provisional Patent Application Serial No. 60/404,932, filed Aug. 21, 2002, U.S. Provisional Patent Application Serial No. 60/370,335, filed Apr. 5, 2002, and U.S. Provisional Patent Application Serial No. 60/361,755 filed Mar. 5, 2002, the entire disclosures of which are specifically incorporated herein by reference.

---------- KWIC ---------

Detail Description Paragraph - DETX (85):

[0188] PI3K has fundamental significance in regulation of diverse cell functions such as growth, survival, and malignant transformation. PI3K itself possesses oncogenic activity as well as the ability to activate a number of other signaling proteins including oncoproteins. The anti-apoptotic effect of

PI3K is realized by activation of proteins from other signaling pathway(s)-protein kinase B (Akt/PKB) and/or PKB-dependent enzymes (GSK-3.beta., ILK-1). PI3K plays a critical role in malignant transformation and can form complexes with some viral or cellular oncoproteins (src, ras, rac, T-antigen, etc.) whose transforming activities are realized only in the presence of PI3K. It has been shown that Ad-mda-7 is directly able to inhibit PI3K function and also suppress the functions of other proto-oncogenes which are regulated by PI3K (Mhashilkar et al., submitted 2002). Ad-mda-7, which encodes a novel tumor suppressor gene may up-regulate other tumor suppressors such as E-cadherin, GSK-3.beta., APC and PTEN. Importantly, Ad-mda-7 transduction in cancer cells is able to potently down-modulate the expression of oncoproteins such as PLC-.gamma., PI3K, Akt, FAK, and .beta.-catenin (FIG. 15). During signal transduction from diverse membrane receptors using growth factor, integrins and other ligands, a complex cascade of molecular events has been described. Ligand-receptor engagement can activate a cascade of PLC-.gamma..fwdarw.FAK.fwdarw.PI3K.fwdarw.Akt, ultimately leading to de novo gene expression. Ad-mda-7 can down-regulate various members of this cascade. The tumor suppressor PTEN can block FAK, PI3K and Akt signaling. Therefore, the activity of Ad-mda-7 on these signaling molecules may be explained by their up-regulation by PTEN. However, Ad-mda-7 may also negatively regulate expression of PLC-.gamma., which is not regulated by PTEN. Thus, MDA-7 appears to function upstream of PLC-.gamma. and PTEN. Ad-mda-7 triggers its anti-proliferative effects in breast and lung cancer cells by activating molecules from the .beta.-catenin and PI3K pathways. It has been shown that oncogenic activation may lead to cross talk between molecules from the .beta.-catenin and PI3K pathways. For example, .beta.-catenin can be stabilized by the p85-.alpha. subunit of PI3K. In addition, cyclin D1, which can be activated by .beta.-catenin stabilization in the nucleus, is regulated by the Wnt-1 and ILK signaling pathways and ILK induction of cyclin D1 involves the CREB signaling pathway in mammary epithelial cells (Woodfield et al., 2001; D'Amico et al., 2000).

PGPUB-DOCUMENT-NUMBER: 20040009604

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040009604 A1

TITLE: Potent oncolytic herpes simplex virus for cancer therapy

PUBLICATION-DATE: January 15, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Zhang, Xiaoliu	Houston	TX	US	
Fu, Xinping	Zhuhai		CN	

APPL-NO: 10/ 397635

DATE FILED: March 26, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60367788 20020327 US

non-provisional-of-provisional 60410024 20020911 US

US-CL-CURRENT: 435/456, 435/235.1 , 435/320.1 , 530/395

ABSTRACT:

The present invention is directed to an oncolytic Herpes Simplex Virus having multiple cell membrane fusion mechanisms and preferably comprising a strict late viral promoter for effective conditional replication, such as in a malignant cell. In specific embodiments, the cell membrane fusion mechanisms are either from a mutant virus generated through random mutagenesis or through insertion of a fusogenic membrane glycoprotein, and in further specific embodiments the strict late viral promoter UL38p regulates expression of the glycoprotein.

[0001] The present invention claims priority to U.S. Provisional Patent Application Serial No. 60/367,788, filed Mar. 27, 2002 and No. 60/410,024, filed Sep. 11, 2002, both of which are incorporated by reference herein in their entirety.

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Detail Description Table CWU - DETL (3):
3TABLE 3 Oncogenes Gene Source Human Disease Function Growth Factors.sup.1 HST/KS Transfection FGF family member INT-2 MMTV promoter FGF family member Insertion INTI/WNTI MMTV promoter Factor-like Insertion SIS Simian sarcoma PDGF B virus Receptor Tyrosine Kinases.sup.1,2 ERBB/HER Avian erythro- Amplified, EGF/TGF-.alpha./ blastosis virus; deleted squamous amphiregulin/ ALV promoter cell cancer; hetacellulin insertion; glioblastoma receptor amplified human tumors ERBB-2/ Transfected from Amplified breast, Regulated by NDF/ NEU/HER-2 rat Glioblatoms ovarian, gastric heregulin and

EGF- cancers related factors FMS SM feline CSF-1 receptor sarcoma virus KIT HZ feline MGF/Steel receptor sarcoma virus hematopoieis TRK Transfection from NGF (nerve growth human colon factor) receptor cancer MET Transfection from Scatter factor/HGF human osteo- receptor sarcoma RET Translocations Sporadic thyroid Orphan receptor Tyr and point cancer; familial kinase mutations medullary thyroid cancer; multiple endocrine neoplasias 2A and 2B ROS URII avian Orphan receptor sarcoma Virus Tyr kinase PDGF Translocation Chronic TEL (ETS-like receptor myclomonocytic transcription factor)/ leukemia PDGF receptor gene fusion TGF-.beta. Colon carcinoma receptor mismatch mutation target NONRECEPTOR TYROSINE KINASES.sup.1 ABI. Abelson Mul.V Chronic Interact with RB, myelogenous RNA polymerase, leukemia CRK, CBL translocation with BCR FPS/FES Avian Fujinami SV; GA FeSV LCK Mul.V (murine Src family; T cell leukemia virus) signaling; interacts promoter CD4/CD8 T cells insertion SRC Avian Rous Membrane- sarcoma Virus associated Tyr kinase with signaling function; activated by receptor kinases YES Avian Y73 virus Src family; signaling SER/THR PROTEIN KINASES.sup.1 AKT AKT8 murine Regulated by retrovirus PI(3)K?; regulate 70-kd S6 k? MOS Maloney murine GVBD; cystostatic SV factor; MAP kinase kinase PIM-1 Promoter insertion Mouse RAF/MIL 3611 murine SV; Signaling in RAS MH2 avian SV pathway MISCELLANEOUS CELL SURFACE.sup.1 APC Tumor suppressor Colon cancer Interacts with catenins DCC Tumor suppressor Colon cancer CAM domains E-cadherin Candidate tumor Breast cancer Extracellular Suppressor homotypic binding; intracellular interacts with catenins PTC/NBCCS Tumor suppressor Nevoid basal cell 12 transmembrane and Drosophilia cancer syndrome domain; signals homology (Gorline through Gli syndrome) homogue Cl to antagonize hedgehog pathway TAN-1 Notch T-ALI. Signaling? homologue Translocation MISCELLANEOUS SIGNALING.sup.1,3 BCL-2 Translocation B-cell lymphoma Apoptosis CBL Mu Cas NS-1 V Tyrosine- phosphorylated RING finger interact Ab1 CRK CT1010 ASV Adapted SH2/SH3 interact Ab1 DPC4 Tumor suppressor Pancreatic cancer TGF-.beta.-related signaling pathway MAS Transfection and Possible angiotensin Tumorigenicity receptor NCK Adaptor SH2/SH3 GUANINE NUCLEOTIDE EXCHANGERS AND BINDING PROTEINS.sup.3,4 BCR Translocated with Exchanger; protein ABL in CML kinase DBL Transfection Exchanger GSP NF-1 Hereditary tumor Tumor suppressor RAS GAP Suppressor neurofibromatosis OST Transfection Exchanger Harvey- HaRat SV; Ki Point mutations Signal cascade Kirsten, RaSV; Balb- in many human N-RAS MoMuSV; tumors Transfection VA V Transfection S112/S113; exchanger NUCLEAR PROTEINS AND TRANSCRIPTION FACTORS.sup.1,5-9 BRCA1 Heritable Mammary Localization suppressor cancer/ovarian unsettled cancer BRCA2 Heritable Mammary cancer Function unknown suppressor ERBA Avian erythro- thyroid hormone blastosis Virus receptor (transcription) ETS Avian E26 virus DNA binding EVII MuLV promotor AML Transcription factor Insertion FOS FBI/FBR murine 1 transcription osteosarcoma factor with c-JUN viruses GLI Amplified glioma Glioma Zinc finger; cubitus interruptus homologue is in hedgehog signaling pathway; inhibitory link PTC and hedgehog HMGG/LIM Translocation Lipoma Gene fusions high t(3:12) t(12:15) mobility group HMGI-C (XT-hook) and transcription factor LIM or acidic domain JUN ASV-17 Transcription factor AP-1 with FOS MLL/ Translocation/ Acute myeloid Gene fusion of VHRX + fusion ELL with leukemia DNA-binding and ELI/MEN MLL Trithorax- methyl transferase like gene MLL with ELI RNA pol II elongation factor MYB Avian myelo- DNA binding blastosis Virus MYC Avian MC29; Burkitt's DNA binding with Translocation lymphoma MAX partner; B-cell cyclin regulation; Lymphomas; interact RB?; promoter regulate apoptosis? Insertion avian leukosis Virus N-MYC Amplified Neuroblastoma L-MYC Lung cancer REL Avian NF-.kappa.B family Retriculoendo- transcription factor theliosis Virus SKI Avian SKV770 Transcription factor Retrovirus VHL Heritable Von Hippel- Negative regulator suppressor Landau syndrome or elongin; transcriptional elongation complex WT-1 Wilm's tumor Transcription factor CELL CYCLE/DNA DAMAGE RESPONSE.sup.10-21 ATM Hereditary Ataxia- Protein/lipid kinase disorder

telangiectasia homology; DNA damage response upstream in P53 pathway BCL-2 Translocation Follicular Apoptosis lymphoma FACC Point mutation Fanconi's anemia group C (predisposition leukemia FHIT Fragile site Lung carcinoma Histidine triad- 3p14.2 related diadenosine 5',3""-P.sup.1.p.sup.4 tetraphosphate asymmetric hydrolase hMLI/MutL HNPCC Mismatch repair; MutL homologue hMSH2/ HNPCC Mismatch repair; MutS MutS homologue

PGPUB-DOCUMENT-NUMBER: 20030229906

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030229906 A1

TITLE: Methods and compositions for the treatment of disorders of HIV infection

PUBLICATION-DATE: December 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Gelman, Irwin H.	Buffalo	NY	US	
Klotman, Paul	New York	NY	US	
Zhou, Ming Ming	Old Greenwich	CT	US	

APPL-NO: 10/ 413785

DATE FILED: April 14, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60372557 20020415 US

US-CL-CURRENT: 800/8, 424/145.1 , 435/325 , 435/456 , 435/5

ABSTRACT:

The present invention relates to methods and compositions for use in the intervention of diseases associated with HIV infection. In exemplary embodiments, methods and compositions for the treatment of HIV associated nephropathy (HIVAN) are disclosed.

[0001] The present application claims the benefit of priority of U.S. Provisional Application No. 60/372,557, which was filed on Apr. 15, 2002 and is incorporated herein by reference in its entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX (103):

[0143] Coding regions for use in constructing the transgenic mice include are HIV-1 Nef, however, it is contemplated that transgenic mice also may be constructed using coding regions for one or more of the other accessory proteins of HIV-1. The coding regions may encode a complete polypeptide, or a fragment thereof, as long as the desired function of the polypeptide is retained, i.e., the polypeptide can modulate transcription of at least one gene that is expressed in podocytes during nephropathy or as a result of activation of the Nef/Src family tyrosine kinase signal. The coding regions for use in constructing the transgenes of the present invention further include those containing mutations, including silent mutations, mutations resulting in a more active protein, mutations that result in a constitutively active protein, and mutations resulting in a protein with reduced activity. Inasmuch as Nef mediates the nephropathic response of an animal in response to HIV-1 infection as identified herein, the following discussion is based on an HIV-1 Nef

transgenic mouse, however, it is understood that the teachings provided herein are equally applicable to other disorders in which the Nef/Src interaction is responsible for a disorder associated with HIV-1 infection. Similarly, the discussion also is applicable to other accessory protein encoding transgenes that may also affect nephropathy upstream or downstream of the effect of HIV-1 Nef.

PGPUB-DOCUMENT-NUMBER: 20030224398

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030224398 A1

TITLE: Purification of the leading front of migratory cells

PUBLICATION-DATE: December 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Klemke, Richard L.	San Diego	CA	US	

APPL-NO: 10/ 365961

DATE FILED: February 12, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60356893 20020213 US

US-CL-CURRENT: 435/6, 435/317.1 , 435/325 , 435/4 , 435/7.2 , 514/54

ABSTRACT:

The invention relates generally to methods of modulating cell migration. Included in the methods of the invention are methods of identifying the state of a pseudopodium in cell migration and methods of inducing extension or retraction of a pseudopodia from a cell. The invention also relates to methods of screening for and identifying an agent effective in inducing extension or retraction of a pseudopodium and therefore affecting cell migration. Agents that can modulate cell migration are useful in treatment of conditions in which cell migration plays a role. Such conditions can include wound healing, angiogenesis, and metastasis of a disease from one location to another. Additionally, the invention provides methods of biochemically separating the pseudopodium of a cell from the remainder of the cell body and methods of determining the proteins present in the pseudopodium and cell body. The invention also includes a pseudopodium isolated by the methods of the invention.

RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. .sctn. 119(e) to U.S. Ser. No. 60/356,893, filed Feb. 13, 2002, the entire content of which is incorporated herein by reference.

---------- KWIC ---------

Detail Description Paragraph - DETX (40):

[0064] By the methods of the invention, it was demonstrated that CAS and Crk are specifically assembled during pseudopodia growth and then disassembled during retraction. This process occurred without apparent change in the overall level of CAS tyrosine phosphorylation or FAK activation, which is an upstream activator of CAS (Vuori et al., 1996; Tachibana et al., 1997). One explanation is that Crk couples only to a specific subset of phosphotyrosine residues present in the substrate domain of CAS, which changes during

pseudopodia growth and retraction. CAS/Crk association is mediated through the binding of the SH2 domain of Crk to phosphotyrosine residues present in the substrate domain of CAS (Matsuda et al., 1993). In fact, there are 15 tyrosine residues in this region of CAS that correspond to potential SH2 binding motifs, 9 of which conform to the Crk SH2 recognition sequence YD(V/T)P (Klemke et al., 1998). Alternatively, regulation may occur through serine phosphorylation of CAS (Ma et al., 2001) or phosphorylation of the regulatory tyrosine 221 of Crk, which prevents CAS/Crk coupling in cells (Kain and Klemke, 2001). However, the latter is unlikely as no significant change in Crk tyrosine phosphorylation was detected. The upstream and downstream components that modulate the assembly/disassembly of this molecular scaffold in the pseudopodium are not yet clear, but likely candidates include c-src, PTP-PEST, and PTP-1B (Garton et al., 1996; Liu et al., 1996; Vuori et al., 1996).

PGPUB-DOCUMENT-NUMBER: 20030224360

PGPUB-FILING-TYPE: corrected

DOCUMENT-IDENTIFIER: US 20030224360 A9

TITLE: Interventions to mimic the effects of calorie restriction

PUBLICATION-DATE: December 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Spindler, Stephen R.	Riverside	CA	US	

APPL-NO: 10/ 056749

DATE FILED: January 22, 2002

RELATED-US-APPL-DATA:

child 10056749 A9 20020122

parent continuation-of 09648642 20000825 US GRANTED

parent-patent 6406853 US

child 09648642 20000825 US

parent continuation-in-part-of 09471224 19991223 US ABANDONED

US-CL-CURRENT: 435/6, 435/4

ABSTRACT:

Long term calorie restriction has the benefit of increasing life span. Methods to screen interventions that mimic the effects of calorie restriction are disclosed. Extensive analysis of genes for which expression is statistically different between control and calorie restricted animals has demonstrated that specific genes are preferentially expressed during calorie restriction. Screening for interventions which produce the same expression profile will provide interventions that increase life span. In a further aspect, it has been discovered that test animals on a calorie restricted diet for a relatively short time have a similar gene expression profile to test animals which have been on a long term calorie restricted diet.

---------- KWIC --------

Detail Description Table CWU - DETL (12):

10TABLE 10 mR:VAs decreased by age and returned to control levels by LT-CR GenBank Phenotype Immune System M30903 B lymphocyte kinase (Blk); src-family protein tyrosine kinase; plays important role in B-cell development/activation and immune responses; B-lineage cells U43384 Cytochrome b-245, beta polypeptide (Cybb, cytochrome b558); integral component of the microbicidal oxidase electron transport chain of phagocytic cells, respiratory burst oxidase; phagocytes U10871 Mitogen activated protein kinase 14 (Mapk14); signal transduction, stimulate phosphorylation of transcription

factors; major upstream activator of MAPKAP kinas 2; hematopoietic stem cells 222649 Myeloproliferative leukemia virus oncogene (Mpl); Member of hematopoietic cytokine receptor family, cell cycle regulator, induces proliferation and differentiation of hematopoietic cell lines; hematopoietic precursor cells, platelets and megakaryocytes Y07521 Potassium voltage gated channel, Shaw-related subfamily member 1 (Kcnc1) potassium channels with properties of delayed rectifiers; nervous system, skeletal system, T lymphocytes U87456 Flavin-containing monooxygenase 1 (Fmo1); xenobiotic metabolism; highly expressed in liver, lung, kidney, lower expressed in heart, spleen, testis, brain U40189 Pancreatic polypeptide receptor 1 (Ppyr1), neuropeptide Y receptor, peptide Y receptor; G-protein-coupled receptor; liver, gastrointestinal tract, prostate, neurons endocrine cells Neuron Specific U16297 Cytochrome b-561 (Cyb561); electron transfer protein unique to neuroendocrin secretory vesicles; vectoral transmembrane electron transport; brain D50032 Trans-golgi network protein 2 (Ttgn2); integral membrane protein localized to the trans-Golgi network; involved in the budding of exocytic transport vesicles; brain neurons Liver Specific/Ubiquitous D82019 Basigin (Bsg), CD147, neurothelin; membrane glycoprotein, immunoglobulin superfamily, homology to MHCs, acts as an adhesion molecule or a receptor, near: network formation and tumor progression; embryo, liver and other organs L38990 Glucokinase (Gk), key glycolytic enzyme; liver U50631 Heat-responsive protein 12 (Hrsp12); heat-responsive, phosphorylated protein sequence simularity to Hsp70; liver, kidney U39818 Tuberous sclerosis 2 (Tsc2); mutationally inactivated in some families with tuberoi sclerosis; encodes a large, membrane-associated GTPase activating protein (GA tuberlin); may have a key role in the regulation of cellular growth; ubiquitous

PGPUB-DOCUMENT-NUMBER: 20030219376

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030219376 A1

TITLE: Progression elevated Gene-3 and uses thereof

PUBLICATION-DATE: November 27, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Fisher, Paul B.	Scarsdale	NY	US	

APPL-NO: 10/ 277603

DATE FILED: October 22, 2002

RELATED-US-APPL-DATA:

child 10277603 A1 20021022

parent continuation-of 09052753 19980331 US GRANTED

parent-patent 6472520 US

child 09052753 19980331 US

parent continuation-in-part-of PCT/US98/05793 19980320 US PENDING

child PCT/US98/05793 19980320 US

parent continuation-in-part-of 08821818 19970321 US GRANTED

parent-patent 6146877 US

US-CL-CURRENT: 424/1.49, 424/93.2 , 435/456 , 536/23.2

ABSTRACT:

This invention provides a vector suitable for introduction into a cell, comprising: a) an inducible PEG-3 regulatory region; and b) a gene encoding a product that causes or may be induced to cause the death or inhibition of cancer cell growth. In addition, this invention further provides the above-described vectors, wherein the inducible PEG-3 regulatory region is a promoter. This invention further provides the above-described vectors, wherein the gene encodes an inducer of apoptosis. In addition, this invention provides the above-described vectors, wherein the gene is a tumor suppressor gene. In addition, this invention provides the above-described vectors, wherein the gene encodes a viral replication protein. This invention also provides the above-described vectors, wherein the gene encodes a product toxic to cells or an intermediate to a product toxic to cells. In addition, this invention provides the above-described vectors, wherein the gene encodes a product causing enhanced immune recognition of the cell. This invention further provides the above-described vectors, wherein the gene encodes a product causing the cell to express a specific antigen.

---------- KWIC ---------

Detail Description Paragraph - DETX (461):

[0514] Defining the mechanism underlying the differential expression of PEG-3 as a function of cancer progression, oncogenic transformation and DNA damage. Nuclear run-on assays indicate that PEG-3 expression directly correlates with an increase in the rate of RNA transcription (17). This association is supported by the isolation of a genomic fragment upstream of the 5' untranslated region of the PEG-3 cDNA and demonstration that this sequence linked to a luciferase reporter gene is activated as a function of cancer progression, oncogenic transformation and DNA damage (FIGS. 15, 16 & 17). Additionally, changes in the stability of PEG-3 mRNA may also contribute to differential expression of this gene as a function of cancer progression, oncogene expression and DNA damage. To address this issue mRNA stability (RNA degradation) assays will be performed as described in detail previously (43). Our analysis focuses on the effect of cancer progression (E11-NMT, R1 and R2 cells), oncogenic transformation (Ha-ras, V-src, H5hr1 and HPV-18 transformed CREF cells) and DNA damage (gamma irradiation and MMS-treatment of CREF cells). Appropriate controls, E11, untransformed CREF cells and CREF cells not treated with DNA damaging agents, respectively, and experimental samples will be incubated without additions or in the presence of 5 mg/ml of actinomycin D (in the dark), and 30, 60 and 120 min later, total cellular RNA will be isolated and analyzed for gene expression using Northern hybridization. RNA blots will be quantitated by densitometric analysis using a Molecular Dynamics densitometer (Sunnyvale, Calif.). These straight forward experiments will indicate if the stability of PEG-3 is altered in cells as a direct consequence of spontaneous progression, expression of defined oncogenes or as a consequence of DNA damage.

Detail Description Paragraph - DETX (578):

[0630] Defining the mechanism underlying the differential expression of PEG-3 as a function of cancer progression, oncogenic transformation and DNA damage. Nuclear run-on assays indicate that PEG-3 expression directly correlates with an increase in the rate of RNA transcription (17). This association is supported by the isolation of a genomic fragment upstream of the 5' untranslated region of the PEG-3 cDNA and demonstration that this sequence linked to a luciferase reporter gene is activated as a function of cancer progression, oncogenic transformation and DNA damage. Additionally, changes in the stability of PEG-3 mRNA may also contribute to differential expression of this gene as a function of cancer progression, oncogene expression and DNA damage. To address this issue mRNA stability (RNA degradation) assays will be performed as described in detail previously (32). Our analysis will focus on the effect of cancer progression (E11-NMT, R1 and R2 cells), oncogenic transformation (Ha-ras, V-src, H5hr1 and HPV-18 transformed CREF cells) and DNA damage (gamma irradiation and MMS-treatment of CREF cells). Appropriate controls, E11, untransformed CREF cells and CREF cells not treated with DNA damaging agents, respectively, and experimental samples will be incubated without additions or in the presence of 5 .mu.g/ml of actinomycin D (in the dark), and 30, 60 and 120 min later, total cellular RNA will be isolated and analyzed for gene expression using Northern hybridization. RNA blots will be quantitated by densitometric analysis using a Molecular Dynamics densitometer (Sunnyvale, Calif.) (32). These straight forward experiments will indicate if the stability of PEG-3 is altered in cells as a direct consequence of spontaneous progression, expression of defined oncogenes or as a consequence of DNA damage.

PGPUB-DOCUMENT-NUMBER: 20030195221

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030195221 A1

TITLE: Substituted indolizine-like compounds and methods of use

PUBLICATION-DATE: October 16, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Cai, Guolin	Thousand Oaks	CA	US	
Chau, Jennifer N.	Santa Ana	CA	US	
Dominguez, Celia	Thousand Oaks	CA	US	
Lu, Yuelie	Thousand Oaks	CA	US	
Rishton, Gilbert M.	Malibu	CA	US	

APPL-NO: 10/ 298205

DATE FILED: November 15, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60332447 20011116 US

US-CL-CURRENT: 514/258.1, 514/259.3 , 514/259.31 , 514/303 , 544/263 , 546/118

ABSTRACT:

Selected novel substituted indolizine-like compounds are effective for treatment of diseases, such as TNF-.alpha., IL-1.beta., IL-6 and/or IL-8 mediated diseases, and other maladies, such as cancer, pain and diabetes. The invention encompasses novel compounds, analogs, prodrugs and pharmaceutically acceptable salts thereof, pharmaceutical compositions and methods for treatment of diseases and other maladies or conditions involving inflammation, cancer, pain, diabetes and the like. The subject invention also relates to processes for making such compounds as well as to intermediates useful in such processes.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (16):

[0015] GB 2,306,108, which is incorporated herein by reference in its entirety, describes imidazole derivatives which are Raf kinase antagonists useful in the treatment of cancer which is mediated by Raf and Raf-inducible proteins. Raf proteins are kinases activated in response to extracellular mitogenic stimuli such as PDGF, EGF, acidic FGF, thrombin, insulin or endothelin, and also in response to oncoproteins such as v-src, v-sis, and v-fms. Raf functions downstream of ras in signal transduction from the cellular membrane to the nucleus. Compounds may be oncolytics through the antagonism of Raf kinase. It has been reported that antisense constructs which reduce cellular levels of c-Raf and hence Raf activity inhibit the growth of rodent fibroblasts in soft agar, while exhibiting little or no general cytotoxicity. This inhibition of growth in soft agar is highly predictive of tumor responsiveness in whole animals. Moreover, Raf antisense constructs have

shown efficacy in reducing tumor burden in animals. Examples of cancers where Raf kinase is implicated by overexpression include cancers of the brain, larynx, lung, lymphatic system, urinary tract and stomach, including hystocytic lymphoma, lung adenocarcinoma and small cell lung cancers. Other examples include cancers involving overexpression of upstream activators of Raf or Raf-activating oncogenes, including pancreatic and breast carcinoma.

PGPUB-DOCUMENT-NUMBER: 20030175935

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030175935 A1

TITLE: Method of identifying inhibitors of Lck

PUBLICATION-DATE: September 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Borhani, David W.	Worcester	MA	US	
Calderwood, David	Framingham	MA	US	
Dixon, Richard W.	Jefferson	MA	US	
Hirst, Gavin C.	Princeton	MA	US	
Hrnciar, Peter	Lexington	MA	US	
Loew, Andreas	Worcester	MA	US	
Leung, Adelaine	Mississauga		CA	
Ritter, Kurt	Frankfurt		DE	

APPL-NO: 10/ 212346

DATE FILED: August 5, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60310051 20010803 US

US-CL-CURRENT: 435/226, 514/252.16 , 514/263.4 , 514/265.1 , 702/19

ABSTRACT:

The present invention relates to polypeptides which comprise the ligand binding domain of Lck, crystalline forms of these polypeptides, and the use of these crystalline forms to determine the three dimensional structure of the catalytic domain of Lck. The invention also relates to the use of the three dimensional structure of the Lck catalytic domain both alone, or in complex with inhibitors, in methods of designing and/or identifying potential inhibitors of Lck activity, for example, compounds which inhibit the binding of a native substrate to the Lck catalytic domain. The invention also relates to the use of the three dimensional structure of the Lck catalytic domain both alone, or in complex with inhibitors, in methods of designing and/or identifying potential selective inhibitors of Lck activity, for example, compounds which inhibit the binding of a native substrate to the Lck catalytic domain selectively.

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 60/310,051 filed Aug. 3, 2001.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (3):
[0003] Lck (lymphocyte cell kinase), a Src-family protein tyrosine kinase expressed primarily in T-cells, plays an essential role in the immune response.

Crucially, Lck is upstream of calcineurin in the TcR signaling cascade. Productive antigen-induced T-cell activation is characterized by the appearance of a Lck-driven, hyperphosphorylated TcR .zeta. chain and by phosphorylation-dependent catalytic activation of the Syk-family kinase ZAP-70 by Lck after docking of ZAP-70 tandem SH2 domains to the phosphorylated amino acids (ITAM motif) in the .zeta. chain. Activated ZAP-70 phosphorylates several substrates that serve as adapter proteins for binding of downstream signaling molecules. This signaling cascade culminates in transcriptional activation of genes involved in cytokine release (particularly IL-2), and ultimately in T-cell clonal expansion in response to an autocrine growth pathway as a prelude to raising an immune response.

PGPUB-DOCUMENT-NUMBER: 20030172388

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030172388 A1

TITLE: Methods and compositions relating to fortilin, an anti-apoptotic molecule, and modulators of fortilin

PUBLICATION-DATE: September 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Fujise, Ken	Houston	TX	US	
Yeh, Edward T.H.	Houston	TX	US	

APPL-NO: 10/ 021753

DATE FILED: October 30, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60244416 20001030 US

US-CL-CURRENT: 800/10, 424/155.1

ABSTRACT:

The polypeptide Fortilin (also known as Translationally Controlled Tumour Protein, TCTP) specifically interacts with p53, a tumor suppressor involved in the induction of apoptosis and the normal growth regulation of a cell. Fortilin also specifically binds MCL1 (Myeloid Cell Leukemia 1). Fortilin has the ability to prevent apoptosis, which may be unregulated in hyperproliferative cells. The present invention is directed at compositions and methods involving a Fortilin modulator, which can induce apoptosis, for the prevention, treatment, or diagnosis of hyperproliferative diseases and conditions, including cancer and atherosclerosis. It is directed also at compositions and methods involving Fortilin, which can inhibit apoptosis, for the treatment of diseases and condition characterized by apoptosis, including certain vascular conditions.

[0001] The present application claims priority to co-pending U.S. Provisional Patent Application Serial No. 60/244,416 filed on Oct. 30, 2000. The entire text of the above-referenced disclosure is specifically incorporated herein by reference without disclaimer. The government may own rights in the present invention pursuant to grant number 1KO8HL04015 from the National Institutes of Health.

---------- KWIC ---------

Detail Description Table CWU - DETL (9):

9TABLE 9 Oncogenes Gene Source Human Disease Function Growth Factors HST/KS Transfection FGF family member INT-2 MMTV promoter FGF family member Insertion INTI/WNTI MMTV promoter Factor-like Insertion SIS Simian sarcoma virus PDGF B Receptor Tyrosine Kinases ERBB/HER Avian erythroblastosis

Amplified, deleted EGF/TGF-.alpha./ virus; ALV promoter Squamous cell Amphiregulin/ insertion; amplified Cancer; glioblastoma Hetacellulin receptor human tumors ERBB-2/NEU/HER-2 Transfected from rat Amplified breast, Regulated by NDF/ Glioblastomas Ovarian, gastric Heregulin and EGF- cancers Related factors FMS SM feline sarcoma virus CSF-1 receptor KIT HZ feline sarcoma virus MGF/Steel receptor Hematopoieis TRK Transfection from NGF (nerve growth human colon cancer Factor) receptor MET Transfection from Scatter factor/HGF human osteosarcoma Receptor RET Translocations and point Sporadic thyroid cancer; Orphan receptor Tyr mutations familial medullary Kinase thyroid cancer; multiple endocrine neoplasias 2A and 2B ROS URII avian sarcoma Orphan receptor Tyr Virus Kinase PDGF receptor Translocation Chronic TEL(ETS-like Myelomonocytic transcription factor)/ Leukemia PDGF receptor gene Fusion TGF-.beta. receptor Colon carcinoma mismatch mutation target NONRECEPTOR TYROSINE KINASES ABI. Abelson Mul. V Chronic myelogenous Interact with RB, RNA leukemia translocation polymerase, CRK, with BCR CBL FPS/FES Avian Fujinami SV; GA FeSV LCK Mul. V (murine leukemia Src family; T cell virus) promoter signaling; interacts insertion CD4/CD8 T cells SRC Avian Rous sarcoma Membrane-associated Virus Tyr kinase with signaling function; activated by receptor kinases YES Avian Y73 virus Src family; signaling SER/THR PROTEIN KINASES AKT AKT8 murine retrovirus Regulated by PI(3)K?; regulate 70-kd S6 k? MOS Maloney murine SV GVBD; cystostatic factor; MAP kinase kinase PIM-1 Promoter insertion Mouse RAF/MIL 3611 murine SV; MH2 Signaling in RAS avian SV Pathway MISCELLANEOUS CELL SURFACE APC Tumor suppressor Colon cancer Interacts with catenins DCC Tumor suppressor Colon cancer CAM domains E-cadherin Candidate tumor Breast cancer Extracellular homotypic Suppressor binding; intracellular interacts with catenins PTC/NBCCS Tumor suppressor and Nevoid basal cell cancer 12 transmembrane Drosophilia homology syndrome (Gorline domain; signals syndrome) through Gli homogue Cl to antagonize hedgehog pathway TAN-1 Notch Translocation T-ALI. Signaling homologue MISCELLANEOUS SIGNALING BCL-2 Translocation B-cell lymphoma Apoptosis CBL Mu Cas NS-1 V Tyrosine-Phosphorylated RING finger interact Abl CRK CT1010 ASV Adapted SH2/SH3 interact Abl DPC4 Tumor suppressor Pancreatic cancer TGF-.beta.-related signaling Pathway MAS Transfection and Possible angiotensin Tumorigenicity Receptor NCK Adaptor SH2/SH3 GUANINE NUCLEOTIDE EXCHANGERS AND BINDING PROTEINS BCR Translocated with ABL Exchanger; protein in CML Kinase DBL Transfection Exchanger GSP NF-1 Hereditary tumor Tumor suppressor RAS GAP Suppressor neurofibromatosis OST Transfection Exchanger Harvey-Kirsten, N-RAS HaRat SV; Ki RaSV; Point mutations in many Signal cascade Balb-MoMuSV; human tumors Transfection VAV Transfection S112/S113; exchanger NUCLEAR PROTEINS AND TRANSCRIPTION FACTORS BRCA1 Heritable suppressor Mammary Localization unsettled cancer/ovarian cancer BRCA 2 Heritable suppressor Mammary cancer Function unknown ERBA Avian erythroblastosis Thyroid hormone Virus receptor (transcription) ETS Avian E26 virus DNA binding EVII MuLV promotor AML Transcription factor Insertion FOS FBI/FBR murine Transcription factor osteosarcoma viruses with c-JUN GLI Amplified glioma Glioma Zinc finger; cubitus interruptus homologue is in hedgehog signaling pathway; inhibitory link PTC and hedgehog HMGI/LIM Translocation t(3:12) Lipoma Gene fusions high t(12:15) mobility group HMGI-C (XT-hook) and transcription factor LIM or acidic domain JUN ASV-17 Transcription factor AP-1 with FOS MLL/VHRX + Translocation/fusion Acute myeloid leukemia Gene fusion of DNA-ELI/MEN ELL with MLL binding and methyl Trithorax-like gene transferase MLL with ELI RNA pol II elongation factor MYB Avian myeloblastosis DNA binding Virus MYC Avian MC29; Burkitt's lymphoma DNA binding with Translocation B-cell MAX partner; cyclin Lymphomas; promoter regulation; interact Insertion avian RB?; regulate leukosis apoptosis? Virus N-MYC Amplified Neuroblastoma L-MYC Lung cancer REL Avian NF-.kappa.B family Retriculoendotheliosis transcription factor Virus SKI Avian SKV770 Transcription factor Retrovirus VHL Heritable suppressor Von Hippel-Landau

Negative regulator or syndrome elongin; transcriptional elongation complex WT-1 Wilm's tumor Transcription factor CELL CYCLE/ DNA DAMAGE RESPONSE ATM Hereditary disorder Ataxia-telangiectasia Protein/lipid kinase homology; DNA damage response upstream in P53 pathway BCL-2 Translocation Follicular lymphoma Apoptosis FACC Point mutation Fanconi's anemia group C (predisposition leukemia FHIT Fragile site 3p14.2 Lung carcinoma Histidine triad-related diadenosine 5',3""- P.sup.1.p.sup.4tetraphosphate asymmetric hydrolase hMLI/MutL HNPCC Mismatch repair; MutL Homologue HMSH2/MutS HNPCC Mismatch repair; MutS Homologue HPMS1 HNPCC Mismatch repair; MutL Homologue hPMS2 HNPCC Mismatch repair; MutL Homologue INK4/MTS1 Adjacent INK-4B at Candidate MTS1 p16 CDK inhibitor 9p21; CDK complexes suppressor and MLM melanoma gene INK4B/MTS2 Candidate suppressor p15 CDK inhibitor MDM-2 Amplified Sarcoma Negative regulator p53 p53 Association with SV40 Mutated >50% human Transcription factor; T antigen tumors, including checkpoint control; hereditary Li-Fraumeni apoptosis syndrome PRAD1/BCL1 Translocation with Parathyroid adenoma; Cyclin D Parathyroid hormone B-CLL or IgG RB Hereditary

PGPUB-DOCUMENT-NUMBER: 20030144204

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030144204 A1

TITLE: Akt-based inducible survival switch

PUBLICATION-DATE: July 31, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Spencer, David	Houston	TX	US	

APPL-NO: 10/ 324985

DATE FILED: December 19, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60342155 20011219 US

US-CL-CURRENT: 514/12, 435/320.1 , 435/325 , 514/44 , 530/350 , 536/23.5

ABSTRACT:

The present invention relates to the field of apoptosis and programmed cell-death. More particularly, it relates to expression vectors, pharmaceutical compositions and methods for inhibiting cell-death using the expression vectors and/or pharmaceutical compositions. Yet further, the present invention also relates to methods of using the expression vector to screen for additional regulators of an anti-apoptotic gene.

[0001] This application claims priority to U.S. Provisional Application serial No. 60/342,155 filed on Dec. 19, 2001.

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Summary of Invention Paragraph - BSTX (10):

[0011] In order to elucidate the function of many signaling molecules, constitutively active or "dominant negative" mutant proteins are often overexpressed in target cells. When Akt or many other "upstream" signaling molecules are modified to contain a membrane-targeting sequence, the increased proximity to activating kinases, such as PDK1, or to membrane-localized substrates typically leads to the constitutive phenotype. For example, most functional Akt studies have utilized either Src family myristoylation-targeting peptides or the myristoylated gag sequence within v-Akt. Under these conditions, however, the kinase is activated as soon as it is expressed in cells, but the effects of activation may not be monitored until much later, when the direct effects of Akt are typically obscured. For controlled gene expression or kinase activation, several approaches are available such as tetracycline-regulatable transcription systems (Gossen et al., 1995), chimeras of hormone binding domains (HBD) with target proteins (Jackson et al., 1993; Picard, 1994; Samuels et al., 1993) and chemically induced dimerization (CID) (Spencer et al., 1993; Spencer, 1996).

PGPUB-DOCUMENT-NUMBER: 20030138905

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030138905 A1

TITLE: Compositions isolated from bovine mammary gland and methods for their use

PUBLICATION-DATE: July 24, 2003

INVENTOR-INFORMATION:

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Grigor, Murray R.	Auckland		NZ	
Molenaar, Adrian J.	Hamilton		NZ	

APPL-NO: 10/ 263828

DATE FILED: October 2, 2002

RELATED-US-APPL-DATA:

child 10263828 A1 20021002

parent continuation-in-part-of 09644265 20000822 US ABANDONED

non-provisional-of-provisional 60150330 19990823 US

US-CL-CURRENT: 435/69.1, 435/198 , 435/320.1 , 435/325 , 530/350 , 530/360 , 536/23.2

ABSTRACT:

Isolated polynucleotides encoding polypeptides expressed in bovine mammary gland tissue are provided, together with genetic constructs and host cells comprising such isolated polynucleotides. Methods for the use of such polynucleotides and polypeptides are also provided.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 09/644,265, filed Aug. 22, 2000, which claims priority to U.S. Provisional Patent Application No. 60/150,330, filed Aug. 23, 1999.

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Summary of Invention - Table CWU - BSTL (1):
1TABLE 1 SEQ SEQ ID NO: ID NO: Poly- Poly- nucleotide peptide Category Related gene function or protein class 1 69 Cell signaling/ Hepatocellular carcinoma and breast cancer associated Differentiation protein. 2 70 Cell signaling/Cell Platelet glycoprotein IV, CD36 antigen or PAS-4 protein, surface antigen has numerous potential physiological functions. It binds to collagen, thrombospondin, anionic phospholipids and oxidized LDL, and functions as a cell adhesion molecule. Directly mediates cytoadherence of Plasmodium falciparum parasitized erythrocytes, binds long chain fatty acids

and functions in the transport and/or as a regulator of fatty acid transport. 3 71 Cell signaling/Cell T-cell receptor T3 delta chain is part of the CD3 complex surface antigen that mediates signal transduction. 4 72 Cell signaling/ Entactin-2 or nidogen-2, is a cell adhesion glycoprotein Differentiation that is involved in osteoblast differentiation. It has a role in cell- extracellular matrix interactions. 5 73 Cell signaling/ Myotrophin, granule cell differentiation protein or v-1 Differentiation protein has a role in cerebellar morphogenesis and functions in differentiation of cerebellar neurons, particularly of granule cells. It is normally expressed at the completion of differentiation and migration of granular cells and at the initiation of the formation of synapses in cerebellar neurons. 6 74 Cell signaling/ Novel abundant testis protein TEGT Differentiation 7 75 Cell signaling/ Connective tissue growth factor is the major connective Growth factor tissue mitoattractant secreted by human vascular endothelial cells. This immediate-early protein binds one of the PDGF cell surface receptors. It belongs to the insulin-like growth factor binding protein family. 8 76 Intracellular cell Adenomatosis polyposis coli binding protein EB1 (APC-signaling binding) is involved in the mechanism through which APC suppresses colonic neoplasia. 9 77 Intracellular cell Adenylyl cyclase-associated protein 1 (CAP 1), is a signaling mammalian homologue of the yeast protein that associates with adenylyl cyclase and thereby enables adenylyl cyclase to respond appropriately to upstream regulatory signals. 10 78 Intracellular cell AMSH - associated molecule with the SH3 domain of signaling STAM (signal transducing adaptor molecule), which is tyrosine-phosphorylated downstream of Jak2 and Jak3 after stimulation with various cytokines such a IL-2, IL-3, IL-4, IL-7, GM- CSF, EGF and PDGF. STAM contains an SH3 (Src-homology 3) domain and the ITAM (immunoreceptor tyrosine-based activation motif), and therefore STAM acts as an adaptor molecule involved in signal transducing pathways from the cytokine receptors. AMSH appears to play a critical role in the cytokine- mediated intracellular signal transduction downstream of the Jak2/Jak3.STAM complex. 11 79 Intracellular cell Nel-like protein 2, is a homotrimer that binds to PKC signaling beta-1. It contain several protein motifs including a secretion signal peptide, an NH(2)-terminal thrombospondin-1 (TSP-1)-like module, five von Willebrand factor C domains, and six epidermal growth factor-like domains. It is strongly expressed in early embryonic neural tissues (brain, spinal cord, and dorsal root ganglia); less in other tissues such as cells around cartilage, myocardium, lung mesenchymal cells, and liver. 12 80 Intracellular cell Peroxisomal targeting signal 2 receptor binds to the N- signaling terminal PTS2-type peroxisomal targeting signal and plays an essential role in peroxisomal protein import. Interacts with PEX5. 13 81 Intracellular cell Peroxisome proliferator-activated receptor binding signaling protein, also known as thyroid hormone receptor- associated protein complex component, TRAP 220, thyroid receptor interacting protein 2, TRIP2, or p53 regulatory protein, RB 18a, interacts with thyroid hormone receptors. to regulate nuclear receptor-mediated transcription. The protein binds DNA and p53 protein. 14 82 Intracellular cell Retinoblastoma binding protein identified as a signaling differentially expressed gene in activation of the transforming growth factor-beta signal transduction pathway in mammary carcinomas treated with the anticancer monoterpenes 15 83 Intracellular cell Silencer of death domains (SODD), is a widely signaling expressed, approximately 60-kilodalton protein, that was found to be associated with the death domain of TNF-R1. TNF treatment released SODD from TNF-RI, permitting the recruitment of proteins such as TRADD and TRAF2 to the active TNF-R1 signaling complex. SODD also interacts with death receptor-3 (DR3), another member of the TNF receptor superfamily and SODD association may be representative of a general mechanism for preventing spontaneous signaling by death domain-containing receptors. 16 84 Cell signaling/ Pigment epithelium-derived factor is a neurotrophic Differentiation protein that

induces extensive neuronal differentiation in retinoblastoma cells. Although it belongs to the serpin family, it does not undergo the S (stressed) to R (relaxed) conformational transition characteristic of active serpins and it exhibits no serine protease inhibitory activity. The N-terminal (aa 42-139) exhibits neurite outgrowth- inducing activity. The C-terminal exposed loop (aa 380- 416) is essential for serpin activity. 17 85 Intracellular cell HSP90 Molecular chaperone with ATPase activity. signaling Belongs to the heat shock protein 90 family. 18 86 Cell signaling/Cell Peanut-like protein 2, brain protein H5, surface antigen is involved in cytokinesis. It belongs to the CDC3/CDC10/CDC11/CDC12 family. 19 87 Cell structure/ Keratin, type I cytoskeletal 17, (K17) is a marker of basal Cytoskeleton cell differentiation in complex epithelia and therefore indicative of a certain type of epithelial "stem cells". There are two types of cytoskeletal and microfibrillar keratin: I (acidic; 40-55 kDa) [K9 to K20] and II (neutral to basic; 56-70 kDa) [K1 to K8]. Both a basic and an acidic keratin are required for filament assembly. Expressed in the hair follicle, nail bed, mucosal stratified squamous epithelia and in basal cells of oral epithelium, palmoplantar epidermis and sweat and mammary glands. K17 is induced in damaged or stressed epidermis. K16 and K17 are coexpressed only in pathological situations such as metaplasias and carcinomas of the uterine cervix and in psoriasis vulgaris. Defects in K17 have been found to be associated with type II pachyonychia congenita (PC) also known as Jackson-Lawler (J&L) syndrome. Type II PC is characterized by onchyogryposis, limited plantar hyperkeratosis, multiple epidermal cysts. 20 88 Cell structure/ Mesothelial keratin K7 (type II) is similar to all other Cytoskeleton intermediate filament proteins. 21 89 Immune Zn-alpha2-glycoprotein appears to be a truncated modulation/MHC secretory major histocompatibility complex-related molecule, and it may have a role in the expression of the immune response. 22 90 Immune Ig lambda C region encodes the light chain of the IgG modulation/ complex which is the major immunoglobulin found in Antigen receptor colostrum and milk of cows. 23 91 Immune Ig gamma-2 chain C region is the heavy chain of the IgG modulation/ complex that is the major antibody class secreted in Antigen receptor colostrum and milk of cows. 24 92 Immune Ig gamma-2 chain C region is the heavy chain of the IgG modulation/ complex that is the major antibody class secreted in Antigen receptor colostrum and milk of cows. 25 93 Metabolic/ GTP cyclohydrolase I feedback regulatory protein Cofactor synthesis mediates tetrahydrobiopterin inhibition of GTP cyclohydrolase I which catalyses the first step in the biosynthesis of tetrahydrofolate. This inhibition is reversed by L-phenylalanine. 26 94 Metabolic/Iron Lactoferrin or lactotransferrin belongs to the transferrin binding family. Transferrins are iron binding transport proteins which can bind two atoms of ferric iron in association with the binding of an anion, usually bicarbonate. Lactoferrin and peptides derived therefrom such as lactoferricin b have antimicrobial properties.

PGPUB-DOCUMENT-NUMBER: 20030138412

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030138412 A1

TITLE: Inhibition of tumor growth and metastasis by N5 gene

PUBLICATION-DATE: July 24, 2003

INVENTOR-INFORMATION:

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Doostzadeh, Jaleh	Fremont	CA	US	

APPL-NO: 10/ 186185

DATE FILED: June 27, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60301619 20010628 US

US-CL-CURRENT: 424/93.21, 435/7.23 , 514/44

ABSTRACT:

The present invention concerns methods for treating pancreatic and ovarian cancers in a subject. These methods employ compositions comprising the N5 gene product, p84N5 and include nucleic acids and proteins/peptides or polypeptides encoding p84N5 or portions thereof. The invention also concerns prognostic applications wherein the levels of expression of p84N5 have been correlated to sensitivity to radiation treatments and/or chemotherapeutic agents. Therefore, the invention also concerns methods for prescribing a specific therapeutic regimen comprising specific radiation and chemotherapy doses and adjustments in such doses based on the individual patients p84N5 expression levels.

[0001] The present application claims priority to co-pending U.S. Application Serial No. 60/301,619 filed Jun. 28, 2001. The entire contents of the above-referenced application are incorporated herein by reference. The government owns rights in the present invention pursuant to grant numbers CA-70292-01 and CA-16672 from the National Institutes of Health.

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Detail Description Table CWU - DETL (5):
5TABLE 4 Gene Source Human Disease Function Growth Factors HST/KS Transfection FGF family member INT-2 MMTV promoter FGF family member Insertion INTI/WNTI MMTV promoter Factor-like Insertion SIS Simian sarcoma virus PDGF B Receptor Tyrosine Kinases ERBB/HER Avian erythroblastosis Amplified, deleted EGF/TGF-.alpha./ virus; ALV promoter squamous cell Amphiregulin/ insertion; amplified cancer; glioblastoma Hetacellulin receptor human tumors ERBB-2/NEU/HER-2 Transfected from rat Amplified breast, Regulated by NDF/ Glioblastomas ovarian, gastric cancers Heregulin and EGF- Related

factors FMS SM feline sarcoma virus CSF-1 receptor KIT HZ feline sarcoma virus MGF/Steel receptor Hematopoieis TRK Transfection from NGF (nerve growth human colon cancer Factor) receptor MET Transfection from Scatter factor/HGF human osteosarcoma Receptor RET Translocations and point Sporadic thyroid cancer; Orphan receptor Tyr mutations familial medullary Kinase thyroid cancer; multiple endocrine neoplasias 2A and 2B ROS URII avian sarcoma Orphan receptor Tyr Virus Kinase PDGF receptor Translocation Chronic TEL(ETS-like Myelomonocytic transcription factor)/ Leukemia PDGF receptor gene Fusion TGF-.beta. receptor Colon carcinoma mismatch mutation target NONRECEPTOR TYROSINE KINASES ABI Abelson Mul.V Chronic myelogenous Interact with RB, RNA leukemia translocation polymerase, CRK, with BCR CBL FPS/FES Avian Fujinami SV; GA FeSV LCK Mul.V (murine leukemia Src family; T-cell virus) promoter signaling; interacts insertion CD4/CD8 T-cells SRC Avian Rous sarcoma Membrane-associated Virus Tyr kinase with signaling function; activated by receptor kinases YES Avian Y73 virus Src family; signaling SER/THR PROTEIN KINASES AKT AKT8 murine retrovirus Regulated by PI(3)K?; regulate 70-kd S6 k? MOS Maloney murine SV GVBD; cystostatic factor; MAP kinase kinase PIM-1 Promoter insertion Mouse RAF/MIL 3611 murine SV; MH2 Signaling in RAS avian SV Pathway MISCELLANEOUS CELL SURFACE.sup.1 APC Tumor suppressor Colon cancer Interacts with catenins DCC Tumor suppressor Colon cancer CAM domains E-cadherin Candidate tumor Breast cancer Extracellular homotypic Suppressor binding; intracellular interacts with catenins PTC/NBCCS Tumor suppressor and Nevoid basal cell cancer 12 transmembrane Drosophilia homology syndrome (Gorline domain; signals syndrome) through Gh homogue Cl to antagonize hedgehog pathway TAN-1 Notch Translocation T-ALI. Signaling? homologue MISCELLANEOUS SIGNALING BCL-2 Translocation B-cell lymphoma Apoptosis CBL Mu Cas NS-1 V Tyrosine-Phosphorylated RING finger interact Abl CRK CT1010 ASV Adapted SH2/SH3 interact Abl DPC4 Tumor suppressor Pancreatic cancer TGF-.beta.-related signaling Pathway MAS Transfection and Possible angiotensin Tumorigenicity Receptor NCK Adaptor 5H2/5H3 GUANINE NUCLEOTIDE EXCHANGERS AND BINDING PROTEINS BCR Translocated with ABL Exchanger; protein in CML Kinase DBL Transfection Exchanger GSP NF-1 Hereditary tumor Tumor suppressor RAS GAP Suppressor neurofibromatosis OST Transfection Exchanger Harvey-Kirsten, N-RAS HaRat SV; K1 RaSV; Point mutations in many Signal cascade Balb-MoMuSV; human tumors Transfection VAV Transfection S112/S113; exchanger NUCLEAR PROTEINS AND TRANSCRIPTION FACTORS BRCA1 Heritable suppressor Mammary Localization unsettled cancer/ovarian cancer BRCA2 Heritable suppressor Mammary cancer Function unknown ERBA Avian erythroblastosis thyroid hormone Virus receptor (transcription) ETS Avian E26 virus DNA binding EVI1 MuLV promotor AML Transcription factor Insertion FOS FBI/FBR murine 1 transcription factor osteosarcoma viruses with c-JUN GLI Amplified glioma Glioma Zinc finger; cubitus interruptus homologue is in hedgehog signaling pathway; inhibitory link PTC and hedgehog HMGI/LIM Translocation t(3:12) Lipoma Gene fusions high t(12:15) mobility group HMGI-C (XT-hook) and transcription factor LIM or acidic domain JUN ASV-17 Transcription factor AP-1 with FOS MLL/VHRX + ELI/MEN Translocation/fusion Acute myeloid leukemia Gene fusion of DNA- ELL with MLL binding and methyl Trithorax-like gene transferase MLL with ELI RNA pol II elongation factor MYB Avian myeloblastosis DNA binding Virus MYC Avian MC29; Burkitt's lymphoma DNA binding with Translocation B-cell MAX partner; cyclin Lymphomas; promoter regulation; interact Insertion avian RB?; regulate leukosis apoptosis? Virus N-MYC Amplified Neuroblastoma L-MYC Lung cancer REL Avian NT-.kappa.B family Retriculoendotheliosis transcription factor Virus SKI Avian SKV770 Transcription factor Retrovirus VHL Heritable suppressor Von Hippel-Landau Negative regulator or syndrome elongin; transcriptional elongation complex WT-1 Wilm's tumor Transcription factor CELL CYCLE/DNA DAMAGE RESPONSE.sup.10-21 ATM Hereditary disorder Ataxia-telangiectasia Protein/lipid kinase homology; DNA damage response upstream in P53 pathway

BCL-2 Translocation Follicular lymphoma Apoptosis FACC Point mutation Fanconi's anemia group C (predisposition leukemia MDA-7 Fragile site 3p14.2 Lung carcinoma Histidine triad-related diadenosine 5',3''''-tetraphosphate asymmetric hydrolase hML1/MutL HNPCC Mismatch repair; MutL Homologue hMSH2/MutS HNPCC Mismatch repair; MutS Homologue hPMS1 HNPCC Mismatch repair; MutL Homologue hPMS2 HNPCC Mismatch repair; MutL Homologue INK4/MTS1 Adjacent INK-4B at Candidate MTS1 p16 CDK inhibitor 9p21; CDK complexes suppressor and MLM melanoma gene INK4B/MTS2 Candidate suppressor p15 CDK inhibitor MDM-2 Amplified Sarcoma Negative regulator p53 p53 Association with SV40 Mutated >50% human Transcription factor; T antigen tumors, including checkpoint control; hereditary Li-Fraumeni apoptosis syndrome PRAD1/BCL1 Translocation with Parathyroid adenoma; Cyclin D Parathyroid hormone B-CLL or IgG RB Hereditary Retinoblastoma; Interact cyclin/cdk; Retinoblastoma; osteosarcoma; breast regulate E2F Association with many cancer; other sporadic transcription factor DNA virus tumor cancers Antigens XPA xeroderma Excision repair; photo- pigmentosum; skin product recognition; cancer predisposition zinc finger

PGPUB-DOCUMENT-NUMBER: 20030134302

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030134302 A1

TITLE: Libraries of expressible gene sequences

PUBLICATION-DATE: July 17, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Hoeffler, James Paul	Carlsbad	CA	US	

APPL-NO: 10/ 210985

DATE FILED: August 1, 2002

RELATED-US-APPL-DATA:

child 10210985 A1 20020801

parent continuation-of 10003021 20011114 US PENDING

child 10003021 20011114 US

parent continuation-of 09285386 19990402 US ABANDONED

non-provisional-of-provisional 60096981 19980818 US

non-provisional-of-provisional 60080626 19980403 US

US-CL-CURRENT: 435/6, 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

The invention described herein comprises libraries of expressible gene sequences. Such gene sequences are contained on plasmid vectors designed to endow the expressed proteins with a number of useful features such as affinity purification tags, epitope tags, and the like. The expression vectors containing such gene sequences can be used to transfect cells for the production of recombinant proteins. A further aspect of the invention comprises methods of identifying binding partners for the products of such expressible gene sequences.

RELATED APPLICATIONS

[0001] This application relies for priority on U.S. Provisional Application No. 60/080,626, filed Apr. 3, 1998, and U.S. Provisional Application No. 60/096,981, filed Aug. 18, 1998, each of which is hereby incorporated herein in its entirety.

---------- KWIC ---------

Detail Description Table CWU - DETL (19):

M221 E6 YLR142W proline oxidase (52.47/60) M84 C2 YLR144C Identified as an activity necessary for actin polymerization in permeabilized cells (85.72/90) M79 E4 YLR009W (22/32) M219 D4 YLR010C (17./6330) M219 D5 YLR011W (21.1/230) M219 D1 YLR015W (55.66/64) M219 D2 YLR016C (22.47/40) M219 D3 YLR017W Protein that regulates ADH2 gene expression (37.18/48) M219 E5 YLR019W (43.78/50) M219 E8 YLR022C (27.53/38) M80 A6 YLR026C Sed5p is a t-SNARE (soluble NSF attachment protein receptor) required in ER to Golgi transport. (37.43/25) M219 F5 YLR027C aspartate aminotransferase cytosolic (47.55/50) M79 F8 YLR029C Ribosomal protein RPL13A (YL10A) (rat L15) (22.47/30) M219 F8 YLR030W (29.04/40) M80 C2 YLR031W (20.57/32) M219 F3 YLR033W (55.33/55) M219 F6 YLR036C (22.46/33) M80 B10 YLR037C (13.67/13) M223 E1 YLR040C (24.67/38) M82 C6 YLR043C thioredoxin (11.46/12) M81 F7 YLR044C pyruvate decarboxylase (61.96/62) M82 D6 YLR051C (23.90/30) M222 G7 YLR053C (11.91/22) M82 C10 YLR054C (56.45/56) M223 B1 YLR055C transcription factor (66.35/70) M81 D2 YLR056W C-5 sterol desaturase (40.36/55) M81 H3 YLR057W (93.5/98) M81 D5 YLR058C serine hydroxymethyltransfe rase (51.62/55) M82 E6 YLR059C (29.62/30) M81 H7 YLR060W Phenylalanyl-tRNA synthetase alpha subunit cytoplasmic (65.56/65) M82 H8 YLR061W 402-755 (13.42/28) M222 A5 YLR066W signal peptidase subunit (20.45/34) M222 H3 YLR073C (22.03/34) M81 E5 YLR074C (18.39/28) M81 E5 YLR074C (18.39/28) M222 A6 YLR075W Ubiquinol- cytochrome C reductase complex subunit VI requiring protein (24.42/33) M222 A6 YLR075W Ubiquinol-cytochrome C reductase complex subunit VI requiring protein (24.42/33) M82 A8 YLR076C (15.43/16) M222 H7 YLR077W (64.24/67) M223 G5 YLR077W (64.24/60) M81 D1 YLR079W P40 inhibitor of Cdc28p-Clb5 protein kinase complex (31.45/50) M223 G3 YLR082C Smc4 protein member of SMC family (43.25/55) M222 B6 YLR083C integral membrane protein.backslash.p24a protein (73.40/90) M222 B4 YLR089C (65.25/65) M222 B4 YLR089C (65.25/65) M81 G5 YLR090W Homolog of E. coli DnaJ closely related to Ydj1p (50.6/60) M81 H6 YLR091W (32.34/40) M81 H6 YLR091W (32.34/40) M222 B8 YLR093C (27.64/39) M223 H2 YLR097C (37.87/52) M81 H5 YLR098C DNA binding activator (71.31/75) M222 D6 YLR099C (43.47/48) M82 C8 YLR100W (38.38/?) M82 C8 YLR100W (38.38/?) M81 A11 YLR102C (29.28/45) M82 F1 YLR103C omosomal DNA replication initiation protein (71.53/?) M222 E6 YLR107W (44.55/48) M222 D8 YLR109W (19.47/38) M81 G4 YLR113W mitogen-activated protein kinase (MAP kinase) (47.96/60) M81 A6 YLR114C (84.07/100) M81 A6 YLR114C (84.07/100) M223 D1 YLR119W suppressor of rnal-1 mutation (23.54/33) M222 D7 YLR124W (12.65/16) M222 F8 YLR125W (15.07/40) M82 A2 YLR127C APC (anaphase promoting complex) component (93.86/94) M82 D7 YLR131C activator of CUP1 expression (84.73/40) M222 E7 YLR132C (31.93/40) M221 A3 YLR137W (40.48/52) M84 C6 YLR139C (70.76/70) M86 G9 YLR141W Upstream activation factor subunit (40.04/55) M221 E6 YLR142W proline oxidase (52.47/60) M84 C2 YLR144C Identified as an activity necessary for actin polymerization in permeabilized cells (85.72/90) M221 F6 YLR150W (30.14/42) M255 H6 YLR151C (37.43/52) M84 G3 YLR153C acetyl-coenzyme A synthetase (75.16/75) M221 G4 YLR155C nitrogen catabolite-regulated cell-wall L- asparaginase II (39.85/50) M221 A2 YLR160C nitrogen catabolite- regulated cell-wall L- asparaginase II (39.85/50) M84 A8 YLR164W (18.59/19) M221 B1 YLR167W ubiquitin (16.83/16) M221 B2 YLR168C (25.33/35) M86 G8 YLR172C S-adenosylmethionine (AdoMet)-dependent methyltransferase of diphthamide biosynthesis (33.03/40) M224 F1 YLR175W major low affinity 55 kDa Centromere.backslash./microtub ule binding protein (53.24/60) M221 C2 YLR176C (89.24/96) M86 H5 YLR178C suppressor of cdc25 (24.12/38) M221 H4 YLR179C (22.14/33) M221 F5 YLR180W S-adenosylmethionine synthetase (42.13/48) M221 E4 YLR186W (27.83/36) M84 A7 YLR187W (112.97/114) M84 D8 YLR188W ATP-binding cassette (ABC) transporter family member (76.56/76) M84 H9 YLR189C (131.81/?) M84 D11 YLR190W (54.12/70) M84 G1 YLR191W Peroxisomal membrane protein that contains Src homology 3 (SH3) domain (42.57/45) M221 F3 YLR193C (19.38/30) M84 B7 YLR195C N-myristoyl transferase (50.08/32) M84 A10 YLR197W homology to

microtubule binding proteins and to X90565_5.cds (55.55/55) M221 D1 YLR199C (24.23/36) M221 E2 YLR200W Polypeptide 6 of a Yeast Non-native Actin Binding Complex homolog of a component of the bovine NABC complex (12.65/18) M84 D4 YLR201C (28.63/40) M84 C7 YLR203C Protein involved in maturation of COX1 and COB mRNA (47.99/48)

PGPUB-DOCUMENT-NUMBER: 20030129752

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030129752 A1

TITLE: Regulation of cytokine production in a hematopoietic cell

PUBLICATION-DATE: July 10, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Gelfand, Erwin W.	Englewood	CO	US	
Johnson, Gary L.	Boulder	CO	US	

APPL-NO: 10/ 193657

DATE FILED: July 10, 2002

RELATED-US-APPL-DATA:

child 10193657 A1 20020710

parent continuation-of 09305720 19990505 US GRANTED

parent-patent 6495331 US

child 09305720 19990505 US

parent continuation-of 08656563 19960531 US GRANTED

parent-patent 5910417 US

US-CL-CURRENT: 435/455, 424/85.2 , 435/372

ABSTRACT:

A method useful for regulating cytokine production by a hematopoietic cell by regulating an MEKK/JNKK-contingent signal transduction pathway in such a cell is disclosed. Methods of identifying compounds capable of specifically regulating an MEKK/JNKK-contingent signal transduction pathway in hematopoietic cells, a kit for identifying cytokine regulators, methods to treat diseases involving cytokine production, and cells useful in such methods are also set forth.

---------- KWIC ---------

Detail Description Paragraph - DETX (32):

[0074] The present inventors have unexpectedly found that the PI3-K inhibitor, wortmannin, at concentrations that inhibit PI3-kinase activity, also inhibited JNK activation, but not ERK activation. This finding is the first demonstration of a role for PI3-kinase in regulating a JNK pathway by an Src family tyrosine kinase-associated receptor. Thus, in mast cells the regulation of the MEKK1, JNKK, JNK pathway is dependent on the activation of PI3-kinase, which in turn, is activated by aggregation of FceRI. Mechanistically, there is a very early separation in the signal pathways activated by the Fc.epsilon.RI

to differentially regulate JNK and ERK sequential protein kinase pathways. Without being bound by theory, the present inventors believe that PI3-kinase activity is involved in activating the MEKK/JNKK-contigent pathway in mast cells downstream of tyrosine kinases and upstream of MEKK1.

US-PAT-NO: 6777439

DOCUMENT-IDENTIFIER: US 6777439 B2

TITLE: Compositions and methods for identifying agents which modulate PTEN function and PI-3 kinase pathways

DATE-ISSUED: August 17, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Durden; Donald L.	Indianapolis	IN	N/A	N/A

APPL-NO: 09/ 870379

DATE FILED: May 30, 2001

PARENT-CASE:

This invention claims priority under 35 U.S.C. .sctn.119(e) to U.S. Provisional Application Nos. 60/208,437 and 60/274,167 filed May 30, 2000 and Mar. 8, 2001 respectively. The entire disclosures of each of the above-identified applications is incorporated by reference herein.

US-CL-CURRENT: 514/456, 514/422

ABSTRACT:

Methods are provided for the identification, biochemical characterization and therapeutic use of agents which impact PTEN, p53, PI-kinase and AKT mediated cellular signaling.

3 Claims, 38 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 25

---------- KWIC ---------

Detailed Description Text - DETX (143):

It is well known that Fc.gamma. receptor crosslinking induces the tyrosine phosphorylation of the adapter protein, Cbl (Park, R. K., et al., (1996) J. Immumology 160:5018). To determine if phagocytic signaling events lead to the phosphorylation of Cbl, the degree of Cbl phosphorylation was assessed before and after induction of phagocytosis. To investigate the role of specific kinases in this phosphorylation event, dominant negative Syk and the Src family kinase inhibitor PP1 were utilized to inhibit the activity of these enzymes. The results demonstrated that Cbl was phosphorylated on tyrosine residues following induction of phagocytosis and this phosphorylation event was abrogated by PP1 (FIGS. 11A and 11B, compare lanes 2-3 to 5-6). This effect was dose dependent (data not shown), as was the effect of PP1 on inhibition of Fc.gamma. receptor-mediated phagocytosis (FIG. 11A). Interestingly, dominant negative Syk inhibited Cbl tyrosine phosphorylation to a lesser extent but completely abrogated the phagocytic response. Interestingly, both PP1 and dominant negative Syk suppressed the basal tyrosine phosphorylation levels of

Cbl in vivo. These data suggested that the catalytic activity of the Src family kinases and the capacity of Syk to dock with the ITAM receptor were both required for Cbl phosphorylation in response to phagocytic stimuli and that these two events were required for phagocytosis. The dominant negative Syk would not be expected to alter the upstream activity of Src family kinases and hence Src mediated phosphorylation of Cbl was not altered to the same extent. The data provided support for a signaling cascade in which Syk functions downstream of Src and upstream of Cbl and other effectors associated with Cbl such as the p85 subunit of PI-3 kinase. The data demonstrated that Src family kinases mediated the phosphorylation of Cbl in a Syk kinase independent manner in vivo. The data also revealed that Src family kinases and Syk were required for phagocytosis mediated by the downstream activation of PI-3 kinase.

US-PAT-NO: 6777415

DOCUMENT-IDENTIFIER: US 6777415 B2

TITLE: Methods of inducing cancer cell death and tumor regression

DATE-ISSUED: August 17, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Daley; George Q.	Weston	MA	02193	N/A

APPL-NO: 09/ 971545

DATE FILED: October 5, 2001

PARENT-CASE:

This application asserts the priority of U.S. provisional application serial No. 60/238,240 filed Oct. 5, 2000.

US-CL-CURRENT: 514/252.14, 514/252.18 , 514/253.01 , 514/290 , 514/908 , 514/922

ABSTRACT:

Methods are provided for treating cancer, comprising administering (1) a farnesyl protein transferase inhibitor in conjunction with (2) an additional Ras signaling pathway inhibitor to induce cancer cell death and tumor regression.

6 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

---------- KWIC ---------

Detailed Description Text - DETX (17):

Another class of signal transduction inhibitors which show an added benefit when combined with farnesyl transferase inhibitors are inhibitors of non-receptor tyrosine kinases. Like receptor tyrosine kinases, non-receptor tyrosine kinases lie upstream in the Ras signal transduction pathway and lead to Ras activation. Unlike receptor tyrosine kinases, non-receptor tyrosine kinases are not localized to the cell membrane, but are soluble proteins localized to the cytoplasm. Examples of these include the src and abl tyrosine kinases. In nearly all patients with chronic myelogenous leukemia, the abl tyrosine kinase is deregulated (i.e. constitutively activated) by a chromosomal translocation in the malignant cells leading to production of the bcr-abl fusion protein.

US-PAT-NO: 6734211

DOCUMENT-IDENTIFIER: US 6734211 B1

TITLE: Compositions and methods for promoting nerve regeneration

DATE-ISSUED: May 11, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gold; Bruce G.	West Linn	OR	N/A	N/A

APPL-NO: 10/ 030904

DATE FILED: April 29, 2002

PARENT-CASE:

This application is a .sctn.371 U.S. national stage of PCT US00/18539 filed Jul. 7, 2000, which was published in English under PCT Article 21(2), which in turn claims the benefit of U.S. Provisional Application No. 60/143,180 filed Jul. 9, 1999.

PCT-DATA:
APPL-NO: PCT/US00/18539
DATE-FILED: July 7, 2000
PUB-NO: WO01/03692
PUB-DATE: Jan 18, 2001
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 514/513

ABSTRACT:

Neurite outgrowth and nerve regeneration are promoted by disruption of the steroid receptor complex and stimulation of MAP kinase/kinase activity. This disruption can take the form of disruption of the physical assembly or function of the steroid receptor complex, such as the mature complex or a precursor of the mature complex that is required for assembly of the mature complex. Geldanamycin and its analogs, bastadin and members of the bastadin family, and radicicol and its analogs, as well as FKBP-52 antibody, are shown to disrupt the complex and promote nerve growth. Assays for finding neurotrophic compounds, as well as compounds found by these assays, pharmaceutical compositions into which they are incorporated, and methods of treating subjects having neuronal dysfunction caused by injury or disease are disclosed. Any of these compounds can be used in combination with a therapeutically effective amount of heat, such as heat applied locally to an area where nerve growth is desired, or systemically in an organism in which neurite growth is desired. Alternatively, these compounds can be used in association with a template, such as a tubular member that defines an anatomic pathway along which nerve regeneration is desired (particularly around a transected or partially transected nerve).

13 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

---------- KWIC ---------

Detailed Description Text - DETX (2):

Steroid receptors are part of a superfamily of molecules that regulate gene expression by direct interaction with the upstream region of specific structural genes. It is essential to hormone action that a receptor must be able to assume both an active and an inactive state. This regulation is accomplished by association of the receptor (the steroid ligand binding component) with a multimeric complex of chaperone proteins, such as heat shock proteins (hsp-90), p23 and FKBP-52, which form the steroid receptor complex (SRC). When the steroid receptor binds its ligand, the receptor is activated, the chaperone proteins of the SRC are dissociated, and a DNA binding domain of the receptor is exposed for interaction with gene regulatory sequences. Members of the steroid receptor family that are regulated in this fashion include mineralocorticoids (such as aldosterone), glucocorticoids (such as dexamethasone), progestins (such as progesterone), androgens (such as testosterone), and estrogens (including estrogen, .beta.-estriol and .beta.-estradiol).

US-PAT-NO: 6639121

DOCUMENT-IDENTIFIER: US 6639121 B1

TITLE: Inducible cancer model to study the molecular basis of host tumor cell interactions in vivo

DATE-ISSUED: October 28, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
DePinho; Ronald A.	Boston	MA	N/A	N/A
Chin; Lynda	Boston	MA	N/A	N/A

APPL-NO: 09/ 619247

DATE FILED: July 19, 2000

PARENT-CASE:

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

This invention was funded with grants received from the National Institutes of Health (Grant Nos. AR02104 and EY11267). Accordingly, the government has certain rights in this invention.

This application claims the benefit of Provisional application Ser. No. 60/146,197, filed Jul. 29, 1999.

US-CL-CURRENT: 800/10, 435/320.1 , 435/325 , 435/455 , 800/18 , 800/3 , 800/8 , 800/9

ABSTRACT:

A non-human mammal having incorporated into its genome an expression construct in which nucleic acid encoding an oncogene is operably linked to an inducible promoter; the mammal further has a genetic mutation that causes it to have an increased susceptibility to cancer.

47 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

---------- KWIC ---------

Detailed Description Text - DETX (17):

Bibliography 1. Bishop, J. M. Molecular themes in oncogenesis. Cell 64, 235-48: (1991). 2. Gossen, M., Freundlieb, S., Bender, G., Muller, G., Hillen, W. & Bujard, H. Transcriptional activation by tetracyclines in mammalian cells. Science 268, 1766-1769 (1995). 3. Fasano, O., Taparowsky, E., Fiddes, J., Wigler, M. & Goldfarb, M. Sequence and structure of the coding region of the human H-ras-1 gene from T24 bladder carcinoma cells. J. Mol. Appl. Genet. 2, 173-180 (1983). 4. Kistner, A., Gossen, M., Zimmermann, F., et al. Doxycycline-mediated quantitative and tissue-specific control of gene

expression in transgenic mice. Proc. Natl. Acad. Sci. U.S.A. 93, 10933-10938 (1996). 5. Chin, L., Pomerantz, J., Polsky, D., et al. Cooperative effects of INK4a and ras in melanoma susceptibility in vivo. Genes Devel. 11, 2822-2834 (1997). 6. Thomson, T. M., Real, F. X., Murakami, S., Cordon-Cardo, C., Old, L. J. & Houghton, A. N. Differentiation antigens of melanocytes and melanoma: analysis of melanosome and cell surface markers of human pigmented cells with monoclonal antibodies. J. Invest. Dermatol. 90, 459-466 (1988). 7. Gause, P. R., Lluria-Prevatt, M., Keith, W. N., et al. Chromosomal and genetic alterations of 7,12-dimethylbenz[a]anthracene-induced melanoma from TP-ras transgenic mice. Mol. Carcin. 20, 78-87 (1997). 8. Shirasawa, S., Furuse, M., Yokoyama, N. & Sasazuki, T. Altered growth of human colon cancer cell lines disrupted at activated Ki-ras. Science 260, 85-88 (1993). 9. Doherty, P. C., Tripp, R. A. & Sixbey, J. W. Evasion of host immune responses by tumours and viruses. Ciba. Found. Symp. 187, 245-256 (1994). 10. Bird, I. N., Spragg, J. H., Ager, A. & Matthews, N. Studies of lymphocyte transendothelial migration: analysis of migrated cell phenotypes with regard to CD31 (PECAM-1), CD45RA and CD45RO. Immunology 80, 553-60: (1993). 11. Traweek, S. T., Kandalaft, P. L., Mehta, P. & Battifora, H. The human hematopoietic progenitor cell antigen (CD34) in vascular neoplasia. Am. J. Clin. Pathol. 96, 25-31: (1991). 12. Rak, J., Filmus, J., Finkenzeller, G., Grugel, S., Marme, D. & Kerbel, R. S. Oncogenes as inducers of tumor angiogenesis. Cancer & Metastasis Reviews 14, 263-277 (1995). 13. Arbiser, J. L., Moses, M. A., Fernandez, C. A., et al. Oncogenic H-ras stimulates tumor angiogenesis by two distinct pathways. Proc. Natl. Acad. Sci. USA 94, 861-866 (1997). 14. Okada, F., Rak, J. W., Croix, B. S., et al. impact of oncogenes in tumor angiogenesis: mutant K-ras up-regulation of vascular endothelial growth factor/vascular permeability factor is necessary, but not sufficient for tumorigenicity of human colorectal carcinoma cells. Proc. Natl. Acad. Sci. U.S.A. 95, 3609-14: (1998). 15. Shweiki, D., Itin, A., Soffer, D. & Keshet, E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature 359, 843-845 (1992). 16. Mazure, N. M., Chen, E. Y., Yeh, P., Laderoute, K. R. & Giaccia, A. J. Oncogenic transformation and hypoxia synergistically act to modulate vascular endothelial growth factor expression. Cancer Res. 56, 3436-3440 (1996). 17. Goldberg, M. A. & Schneider, T. J. Similarities between the oxygen-sensing mechanisms regulating the expression of vascular endothelial growth factor and erythropoietin. J. Biol. Chem. 269, 4355-4359 (1994). 18. Mukhopadhyay, D., Tsiokas, L., Zhou, X. M., Foster, D., Brugge, J. S. & Sukhatme, V. P. Hypoxic induction of human vascular endothelial growth factor expression through c-Src activation. Nature 375, 577-581 (1995). 19. Rak, J., Mitsuhashi, Y., Bayko, L., et al. Mutant ras oncogenes upregulate VEGF/VPF expression: implications for induction and inhibition of tumor angiogenesis. Cancer Res. 55, 4575-4580 (1995). 20. Grugel, S., Finkenzeller, G., Weindel, K., Barleon, B. & Marine, D. Both v-Ha-Ras and v-Raf stimulate expression of the vascular endothelial growth factor in NIH 3T3 cells. J. Biol. Chem. 270, 25915-25919 (1995). 21. Larcher, F., Robles, A. I., Duran, H., et al. Up-regulation of vascular endothelial growth factor/vascular permneability factor in mouse skin carcinogenesis correlates with malignant progression state and activated H-ras expression levels. Cancer Res 56, 5391-5396 (1996). 22. Ganss, R., Montoliu, L., Monaghan, A. P. & Schutz, G. A cell-specific enhancer far upstream of the mouse tyrosinase gene confers high level and copy number-related expression in transgenic mice. EMBO 13, 3083-3093 (1994). 23. Valenzuela, D. M., Stitt, T. N., DiStefano, P. S., et al. Receptor tyrosine kinase specific for the skeletal muscle lineage: expression in embryonic muscle, at the neuromuscular junction, and after injury. Neuron 15, 573-84: (1995). 24. Schreiber-Agus, N., Meng, Y., Hoang, T., et al. Role of Mxi1 in ageing organ systems and the regulation of normal and neoplastic growth. Nature 393, 483-7: (1998). 25. Gavrieli, Y., Sherman, Y. & Ben-Sasson, S. A. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J. Cell Biol. 119,

493-501: (1992). 26. Cheng, L., Fu, J., Tsukamoto, A. & Hawley, R. G. Use of green fluorescent protein variants to monitor gene transfer and expression in mammalian cells. Nat Biotechnol. 14, 606-609 (1996).

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	174	hbx	US-PGPUB; USPAT	OR	OFF	2004/09/21 09:05
L2	492	(hepatitis adj b adj virus or hbv) near4 inhibit$8	US-PGPUB; USPAT	OR	OFF	2004/09/21 09:07
L3	26	1 and 2	US-PGPUB; USPAT	OR	OFF	2004/09/21 09:07
L4	11	hbx near4 inhibit$8	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:02
L5	10160	src	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:08
L6	794441	activat$8	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:08
L7	239593	upstream	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:09
L8	3912	6 near5 7	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:09
L9	27	8 same 5	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:13
L10	97	5 same 6 same 7	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:13
L11	334	5 near2 (activator$1 or activation)	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:20
L12	4205	hbv or hbx	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:20
L13	19	11 and 12	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:20

PGPUB-DOCUMENT-NUMBER: 20040132043

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040132043 A1

TITLE: Proteins Associated with cell growth, differentiation, and death

PUBLICATION-DATE: July 8, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Azimzai, Yalda	Oakland	CA	US	
Au-Young, Janice K	Brisbane	CA	US	
Batra, Sajeev	Oakland	CA	US	
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Burford, Neil	Durham	CT	US	
Ding, Li	Creve Couer	MO	US	
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APPL-NO: 10/ 474291

DATE FILED: October 6, 2003

PCT-DATA:
APPL-NO: PCT/US02/11152
DATE-FILED: Apr 5, 2002
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/6, 435/320.1 , 435/325 , 435/69.1 , 530/350 , 536/23.5

ABSTRACT:

The invention provides human proteins associated with cell growth, differentiation, and death (CGDD) and polynucleotides which identify and encode CGDD. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of CGDD.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (120):
[0118] Ras regulates other signaling pathways by direct interaction with different cellular targets (Katz, M. E. and McCormick, F. (1997) Curr. Opin. Genet. Dev. 7:75-79). One such target is RalGDS, a guanine nucleotide dissociation stimulator for the Ras-like GTPase, Ral (Albright, C. F. et al. (1993) EMBO J. 12:339-347). RalGDS couples the Ras and Ral signaling pathways. Epidermal growth factor (EGF) stimulates the association of RalGDS with Ras in mammalian cells, which activates the GEF activity of RalGDS (Kikuchi, A. and Williams, L. T. (1996) J. Biol. Chem. 271:588-594; Urano, T. et al. (1996) EMBO J. 15:810-816). Ral activation by Ral-GDS leads to activation of Src, a tyrosine kinase that phosphorylates other molecules including transcription factors and components of the actin cytoskeleton (Goi, T. et al. (2000) EMBO J. 19:623-630). Ral interacts with a number of signaling molecules including Ral-binding protein, a GAP for the Rho-like GTPases; Cdc42 and Rac, which regulate cytoskeletal rearrangement; and phospholipase D1, which is involved in vesicular trafficking (Feig, L. A. et al. (1996) Trends Biochem. Sci. 21:438-441; Voss, M. et al. (1999) J. Biol. Chem. 274:34691-34698).

Detail Description Paragraph - DETX (175):
[0352] In another embodiment of the invention, polynucleotides encoding CGDD may be used for somatic or germline gene therapy. Gene therapy may be performed to (i) correct a genetic deficiency (e.g., in the cases of severe combined immunodeficiency (SCID)-X1 disease characterized by X-linked inheritance (Cavazzana-Calvo, M. et al. (2000) Science 288:669-672), severe combined immunodeficiency syndrome associated with an inherited adenosine deaminase (ADA) deficiency (Blaese, R. M. et al. (1995) Science 270:475-480; Bordignon, C. et al. (1995) Science 270:470-475), cystic fibrosis (Zabner, J. et al. (1993) Cell 75:207-216; Crystal, R. G. et al. (1995) Hum. Gene Therapy 6:643-666; Crystal, R. G. et al. (1995) Hum. Gene Therapy 6:667-703), thalassamias, familial hypercholesterolemia, and hemophilia resulting from Factor VIII or Factor IX deficiencies (Crystal, R. G. (1995) Science 270:404-410; Verma, I. M. and N. Somia (1997) Nature 389:239-242)), (ii) express a conditionally lethal gene product (e.g., in the case of cancers which result from unregulated cell proliferation), or (iii) express a protein which affords protection against intracellular parasites (e.g., against human retroviruses, such as human immunodeficiency virus (HIV) (Baltimore, D. (1988) Nature 335:395-396; Poeschla, E. et al. (1996) Proc. Natl. Acad. Sci. USA 93:11395-11399), hepatitis B or C virus (HBV, HCV); fungal parasites, such as Candida albicans and Paracoccidioides brasiliensis; and protozoan parasites such as Plasmodium falciparum and Trypanosoma cruzi). In the case where a genetic deficiency in CGDD expression or regulation causes disease, the expression of CGDD from an appropriate population of transduced cells may alleviate the clinical manifestations caused by the genetic deficiency.

PGPUB-DOCUMENT-NUMBER: 20040110177

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040110177 A1

TITLE: Method for identifying functional nucleic acids

PUBLICATION-DATE: June 10, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 470845

DATE FILED: July 31, 2003

PCT-DATA:
APPL-NO: PCT/EP02/01073
DATE-FILED: Feb 1, 2002
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/6

ABSTRACT:

The present invention relates to a method for identifying nucleic acid molecules functionally associated with a desired phenotype.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (22):
[0022] Alternatively, the desired phenotype may be selected from other properties such as production of secreted protein, e.g. insulin, growth hormone, interferons etc., susceptibility or resistance to pathogens, e.g. viruses such as HCV, HBV or other pathogens, senescence and regulation of cell functions, i.e. the identification of genes that regulate certain cell functions e.g. identification of negative regulators of insulin receptor activity comprising a screen for cell clones with upregulated insulin receptor activity.

Detail Description Paragraph - DETX (150):
[0187] 76. Nada, S., et al., Constitutive activation of Src family kinases in mouse embryos that lack Csk Cell, 1993. 73(6): p. 1125-35.

PGPUB-DOCUMENT-NUMBER: 20040097531

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040097531 A1

TITLE: Inhibitors of c-Jun N-terminal kinases (JNK) and other protein kinases

PUBLICATION-DATE: May 20, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 616560

DATE FILED: July 9, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60395202 20020709 US

US-CL-CURRENT: 514/275, 514/341 , 514/342 , 544/331 , 546/270.4 , 546/271.4 , 546/272.7

ABSTRACT:

The present invention provides compounds of formula I: 1
or a pharmaceutically acceptable derivative thereof, wherein R.sup.1, R.sup.2, A, G, and W are as described in the specification. These compounds are inhibitors of protein kinase, particularly inhibitors of JNK, a mammalian protein kinase involved cell proliferation, cell death and response to extracellular stimuli, Lck, Src, and Aurora kinases. The invention also relates to methods for producing these inhibitors. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application 60/395,202, filed Jul. 9, 2002, which is hereby incorporated by reference in its entirety.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (19):
[0018] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus [Klein et al., EMBO J., 18:5019, (1999) and Klein et al., Mol. Cell. Biol., 17:6427 (1997)].

Summary of Invention Paragraph - BSTX (31):
[0030] There is a continued need to develop potent inhibitors of JNKs, Src

family kinases, and Aurora family kinases that are useful in treating or preventing various conditions associated with JNK, Src, and Aurora activation.

PGPUB-DOCUMENT-NUMBER: 20040077663

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040077663 A1

TITLE: Thienopyrimidine-based inhibitors of the src family

PUBLICATION-DATE: April 22, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 399816

DATE FILED: October 6, 2003

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
US	09694145	2000US-09694145	October 23, 2000

PCT-DATA:
APPL-NO: PCT/US01/50198
DATE-FILED: Oct 23, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 514/260.1, 544/280

ABSTRACT:

Various thienopyrimidine-based analog compounds are able to selectively inhibit the Src family of tyrosine kinases. These compounds are useful in the treatment of various diseases including hyperproliferative diseases, hematologic diseases, osteoporosis, neurological diseases, autoimmune diseases, allergic/immunological diseases, or viral infections.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (12):

[0012] Herpesviridae, papovaviridae, and retroviridae have been shown to interact with non-receptor tyrosine kinases and use them as signaling intermediates. The HIV-1 Nef protein interacts with members of the Src family of tyrosine kinases. Nef mediates downregulation of CD4 membrane expression, modification of T-cell activation pathways, and increases virus infectivity (Collette et al., 1997). The HBx protein of the hepatitis B virus is essential for infection by hepadnaviruses and activates Ras by activating the Src family of tyrosine kinases. The activation of Ras is necessary for the ability of the HBx protein to stimulate transcription and release growth arrest in quiescent cells (Klein and Schneider, 1997). Activity of the Src family of tyrosine kinases is altered by association with viral proteins such as mouse and hamster

polyomavirus middle-T antigens, Epstein-Barr virus LMP2A, and herpesvirus saimiri Tip (Dunant and Ballmer-Hofer, 1997).

Detail Description Paragraph - DETX (218):
[0282] Bolen J B, Veillette A, Schwartz A M, Deseau V, Rosen N: Activation of pp60c-src in human colon carcinoma and normal human colon mucosal cells. Oncogene Res 1: 149-168, 1987.

Detail Description Paragraph - DETX (219):
[0283] Bolen J B, Veillette A, Schwartz A M, Deseau V, Rosen N: Activation of pp60c-src protein kinase activity in human colon carcinoma. Proc Natl Acad Sci USA 84:2251-2255, 1987.

Detail Description Paragraph - DETX (229):
[0293] Cartwright C A, Kamps M P, Meisler A I, Pipas J M, Eckhart W: p60c-src activation in human colon carcinoma. J Clin Invest 83:2025-2033, 1989.

Detail Description Paragraph - DETX (230):
[0294] Cartwright C A, Meisler A I, Eckhart W: Activation of the pp60c-src protein kinase is an early event in colonic carcinogenesis. Proc Natl Acad Sci USA 87:558-562, 1990.

Detail Description Paragraph - DETX (232):
[0296] Chackalaparampil I, Shalloway D: Altered phosphorylation and activation of pp60c-src during fibroblast mitosis. Cell 52:801-810, 1988.

Detail Description Paragraph - DETX (270):
[0334] Klein N P and Schneider R J. Activation of Src Family Kinases by Hepatitis B Virus HBx Protein and Coupled Signaling to Ras. Mol Cell Biol 17:6427-6436, 1997.

Detail Description Paragraph - DETX (296):
[0360] Sabe H, Okada M, Nakagawa H, Hanafusa H: Activation of c-src in cells bearing v-Crk and its suppression by CSK. Mol. Cell Biol. 12:4706-4713, 1992.

Detail Description Paragraph - DETX (328):
[0392] Zheng X M, Wang Y, and Pallen C J: Cell transformation and activation of pp60c-src by overexpression of a protein tyrosine phosphatase. Nature 359:336-339, 1992.

PGPUB-DOCUMENT-NUMBER: 20040023963

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040023963 A1

TITLE: Inhibitors of c-Jun N-terminal kinases (JNK) and other protein kinases

PUBLICATION-DATE: February 5, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 437666

DATE FILED: May 14, 2003

RELATED-US-APPL-DATA:

child 10437666 A1 20030514

parent division-of 10121035 20020410 US PENDING

non-provisional-of-provisional 60283621 20010413 US

non-provisional-of-provisional 60329440 20011015 US

non-provisional-of-provisional 60292974 20010523 US

US-CL-CURRENT: 514/242, 514/227.8 , 514/235.8 , 514/252.01 , 514/275 , 544/112 , 544/182 , 544/238 , 544/331 , 544/60

ABSTRACT:

The present invention provides compounds of formula I: 1
or a pharmaceutically acceptable derivative o thereof, wherein A, B, R.sup.a, R.sup.1, R.sup.2, R.sup.3, and R.sup.4 are as described in the specification. These compounds are inhibitors of protein kinase, particularly inhibitors of JNK, a mammalian protein kinase involved cell proliferation, cell death and response to extracellular stimuli; Lck and Src kinase. The invention also relates to methods for producing these inhibitors. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of priority to co-pending U.S. provisional applications No. 60/283,621 filed Apr. 13, 2001, No. 60/329,440 filed Oct. 14, 2001 and No. 60/292,974 filed May 23, 2001.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (19):
[0018] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J., 18, 5019, (1999) and Klein et al., Mol. Cell. Biol., 17, 6427 (1997).

Summary of Invention Paragraph - BSTX (22):
[0021] Accordingly, there is still a great need to develop potent inhibitors of JNKs and Src family kinases that are useful in treating various conditions associated with JNK and Src activation.

PGPUB-DOCUMENT-NUMBER: 20040001853

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040001853 A1

TITLE: Chimeric antigens for eliciting an immune response

PUBLICATION-DATE: January 1, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 365620

DATE FILED: February 13, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60423578 20021105 US

non-provisional-of-provisional 60390564 20020620 US

US-CL-CURRENT: 424/189.1

ABSTRACT:

Disclosed herein are the nucleotide sequences, deduced amino acid sequences as well as methods and compositions necessary to elicit immune responses against chronic Hepatitis B infections in animals and humans. Immune response is enhanced by fusing relevant viral antigens with xenotypic immunoglobulin heavy chain region through a peptide linker and producing the fusion proteins in Baculovirus expression system to incorporate high mannose glycosylation. By virtue of the antibody component, the fusion proteins bind to Fc receptors on the surface of antigen presenting cells, are taken up, processed and derived peptides are presented on MHC Class I, which elicit a CTL (Th1) response. In a similar fashion, due to cross priming and presentation on MHC Class II, will elicit a humoral (Th2) response. In addition, disclosed are the methods of cloning, expression and production of the fusion proteins.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (2):
[0001] The present invention relates to chimeric antigens (fusion proteins) for targeting and activating antigen presenting cells. In particular, the invention describes compositions and methods that contain or use one or more fusion proteins that contain a pre-selected HBV antigen or an HCV antigen, and a xenotypic immunoglobulin fragment, wherein the fusion molecule is capable of binding and activating antigen presenting cells, especially dendritic cells.

Summary of Invention Paragraph - BSTX (4):
[0002] Viral infectious diseases are major public healthcare issues. Human Hepatitis B virus (HBV) is a member of a family of DNA viruses that primarily infect the liver (Gust, 1986). Other members of this family are woodchuck

hepatitis B virus (WHV) ((Summers, Smolec et al. 1978), duck hepatitis B virus (DHBV) ((Mason, Seal et al. 1980) and heron hepatitis B virus (HHBV) (Sprengel, Kaleta et al. 1988). These viruses share a common morphology and replication mechanisms, but are species specific for infectivity (Marion, 1988).

Summary of Invention Paragraph - BSTX (5):
[0003] HBV primarily infects liver cells and can cause acute and chronic liver disease resulting in cirrhosis and hepatocellular carcinoma. Infection occurs through blood and other body fluids. Approximately 90% of the individuals infected by HBV are able to clear the infection, while the remaining 10% become chronic carriers of the virus with a high probability of developing cirrhosis of the liver and hepatocellular carcinoma. The world Health Organization statistics show that more than 2 billion people have been infected by HBV and among these, 350 million are chronically infected by the virus (Beasley 1988) (Law J Y 1993). Prophylactic vaccines based on HBV surface antigen (HbSAg) have been very effective in providing protective immunity against HBV infections. These vaccines have been developed from HbSAg purified from plasma of chronic HBV carriers, produced by recombinant DNA techniques as well as through the use of synthetic peptides (Please see U.S. Pat. Nos. 4,599,230 and 4,599,231). These vaccines are highly effective in the prevention of infection, but are ineffective in eradicating established chronic infections.

Summary of Invention Paragraph - BSTX (6):
[0004] Human Hepatitis B Virus (HBV) belongs to the family of Hepadnaviruses. Other members of this family are Duck Hepatitis B Virus (DHBV), Woodchuk Hepatitis Virus (WHV) Ground squirrel Hepatitis B Virus (GSHV) and Heron Hepatitis B Virus (HHBV). Although these viruses have similar morphology and replication mechanism, they are fairly species specific consequently, infect only very closely related species. These viruses have a DNA genome ranging in size of 3.0-3.2 Kb, with overlapping reading frames to encode several proteins. HBV genome encodes several proteins. Among these, the surface antigens: Large (S1/S2/S), Medium (S2/S) and Small (S) are proposed to be involved in the binding of the virus to the cellular receptors for uptake. The core protein (Core) forms capsids which encapsulate the partially double stranded DNA genome. Polymerase/Reverse Transcriptase (Pol) protein is a multifunctional enzyme necessary for the replication of the virus. The X protein has been proposed to have many properties, including the activation of Src kinases (Ganem and Schneider, 2001). The present invention describes DNA sequences and amino acid compositions of the surface antigen proteins S1/S2, S1/S2/S as well as Core protein fusion proteins with a xenotypic Mab fragment.

Summary of Invention Paragraph - BSTX (16):
[0014] In patients with chronic viral infections, since the virus is actively replicating, viral antigens will be produced within the host cell. Secreted antigens will be present in the circulation. As an example, in the case of chronic HBV carriers, virions, the HBV surface antigens and the core antigens can be detected in the blood. An effective therapeutic vaccine should be able to induce strong CTL responses against an intracellular antigen or an antigen delivered into the appropriate cellular compartment so as to activate the MHC Class I processing pathway.

Summary of Invention Paragraph - BSTX (19):
[0017] Dendritic cells derived from blood monocytes, by virtue of their capability as professional antigen presenting cells have been shown to have great potential as immune modulators which stimulate primary T cell response (Steinman, Inaba et al. 1999), (Banchereau and Steinman 1998). This unique property of the DCs to capture, process, present the antigen and stimulate naive T cells has made them very important tools for therapeutic vaccine

development (Laupeze, Fardel et al. 1999). Targeting of the antigen to the DCs is the crucial step in the antigen presentation and the presence of several receptors on the DCs for the Fc region of monoclonal antibodies have been exploited for this purpose (Regnault, Lankar et al. 1999). Examples of this approach include ovarian cancer Mab-B43.13, Anti-PSA antibody as well as Anti-HBV antibody antigen complexes (Wen, Qu et al. 1999). Cancer immunotherapy using DCs loaded with tumor associated antigens have been shown to produce tumor-specific immune responses and anti-tumor activity (Campton, Ding et al. 2000; Fong and Engleman 2000). Promising results were obtained in clinical trials in vivo using tumor-antigen-pulsed DCs (Tarte and Klein 1999). These studies clearly demonstrate the efficacy of using DCs to generate immune responses against cancer antigens.

Summary of Invention Paragraph - BSTX (23):

[0020] The present invention relates to chronic infectious diseases, and in particular to chronic HBV infections. The presentation of HBV antigens to elicit a CTL response by the use of vaccine molecules designed to target the vaccines to DCs whereby the HBV-associated antigens treated as "self" during the chronic infection will be recognized as "foreign" and the host's immune system will mount a CTL response to eliminate HBV-infected cells. At the same time, through cross presentation, the antibody response to the circulating HBV antigen will bind to the antigen and remove it from the circulation. Accordingly, the present invention is designed to produce vaccines which can induce a broad immune response in patients who have chronic viral infections such as HBV.

Summary of Invention Paragraph - BSTX (24):

[0021] One or more embodiments of the present invention include one or more chimeric antigens suitable for initiating an immune response against Hepatitis B virus (HBV). In these embodiments of the invention, the nucleotide and deduced amino acid sequences for pre-selected HBV antigens are linked to fragments of xenotypic antibodies. The resulting chimeric antigens are capable of targeting and activating antigen presenting cells, such as dendritic cells.

Brief Description of Drawings Paragraph - DRTX (13):

[0038] FIG. 9 shows the nucleotide and deduced amino acid sequences of the expressed HBV S1/S2 protein.

Brief Description of Drawings Paragraph - DRTX (16):

[0041] FIG. 12 shows the nucleotide and deduced amino acid sequences of the expressed HBV S1/S2/S protein.

Brief Description of Drawings Paragraph - DRTX (19):

[0044] FIG. 15 shows the nucleotide and deduced amino acid sequences of the expressed HBV core protein.

Brief Description of Drawings Paragraph - DRTX (37):

[0062] FIG. 33 shows the nucleotide (A) and amino acid (B) sequences of the ORF of HBV S1/S2-TBD in the plasmid pFastbachta-tbd.

Brief Description of Drawings Paragraph - DRTX (38):

[0063] FIG. 34 shows the comparison of binding of HBV S1/S2-TBD, IgG1, and IgG2 over time.

Brief Description of Drawings Paragraph - DRTX (39):
[0064] FIG. 35 shows the comparison of HBV S1/S2-TBD, IgG1, and IgG2a binding to maturing dendritic cells on day 1.

Brief Description of Drawings Paragraph - DRTX (40):
[0065] FIG. 36 shows the comparison of HBV S1/S2-TBD, IgG1, and IgG2a binding to maturing dendritic cells on day 4.

Brief Description of Drawings Paragraph - DRTX (41):
[0066] FIG. 37 shows the comparison of uptake between HBV S1/S2-TBD, IgG1, and IgG2 as a function of concentration.

Brief Description of Drawings Paragraph - DRTX (42):
[0067] FIG. 38 shows the correlation of HBV S1/S2-TBD to CD32 and CD206 expression on dendritic cells.

Brief Description of Drawings Paragraph - DRTX (43):
[0068] FIG. 39 shows that the binding of HBV S1/S2-TBD to DC32 and DC206 receptors on dendritic cells is abolished by anti-Fc Mab.

Detail Description Paragraph - DETX (3):
[0089] In some embodiments of the invention, the IRD of the chimeric antigen includes one or more proteins selected from the group comprising: one or more HBV proteins, one or more recombinant HBV proteins, one or more HCV proteins, or one or more recombinant HCV proteins.

Detail Description Paragraph - DETX (4):
[0090] In yet another embodiment of the invention, IRD of the chimeric antigen includes 6-His-peptide fused to one or more HBV proteins, one or more recombinant HBV proteins, one or more HCV proteins, or one or more recombinant HCV proteins.

Detail Description Paragraph - DETX (32):
[0118] "Immune Response Domain (IRD)" refers to the variously configured antigenic portion of a bifunctional molecule The IRD comprises one or more antigens or one or more recombinant antigens. Viral antigens may include, but are not limited to, HBV PreS1/S2 HBV PreS1/S2/S, HBV Core, HBV Core ctm .alpha.-terminal modified), HBV e-antigen, HBV Polymerase, HCV Core, HCV E1-E2, HCV E1, HCV E2, HCV NS3-serine protease, HCV NS5A and NS4A, HIV GP120 and HSV Alkaline nuclease and HPV Antigens.

Detail Description Paragraph - DETX (41):
[0127] An embodiment of the present invention includes the use of recombinant antigens of HBV, HCV, or DHBV virus fused to a xenotypic antibody fragment by molecular biological techniques, production of the fusion proteins in baculovirus expression system and their use as therapeutic vaccines against chronic HBV and HCV infections. The present invention provides an efficient method to deliver a hitherto unrecognized antigen to APCs in vivo so as to generate a broad immune response, a Th1 response involving CTLs and a Th2 (antibody) response. The immunogenicity of the pre-selected viral antigen unrecognized by the host immune system is increased due to the presence of the xenotypic antibody fragment as well as by the presence of specific glycosylation introduced in the insect cell expression system. The antigen-antibody fragment fusion protein, due to the presence of the antibody

component, will bind to specific receptors present on various immune cell types including dendritic cells, macrophages, B-cells and granulocytes. The fusion proteins administered to either humans or animals will be taken up by the APCs, especially DCs, will be hydrolysed to small peptides and presented on the cell surface, complexed with MHC Class I and/or MHC Class II, which can elicit a broad immune response and clear the viral infection.

Detail Description Paragraph - DETX (42):
[0128] As used herein, the term "Target Binding Domain (TBD)" refers to the region of an immunoglobulin heavy chain constant region, which is a portion capable of binding to an Fc receptor on an APC. This is derived from Mouse anti-HBVsAg Mab (Hybridoma 2C12) as cloned in pFASTBAC HTa expression vector, and expressed in High Five insect cell expression system (Invitrogen). The constant region of the heavy chain of the immunoglobulin molecule consists of part of CH1, and Hinge-CH2-CH3 from N-terminal to C-terminal. The constant region of the IgG1 molecule for the practice of the present invention contains a linker peptide, part of C.sub.H1-hinge and the regions C.sub.H2 and C.sub.H3. The hinge region portion of the monomeric TBD can form disulphide bonds with a second TBD molecule. FIG. 5 illustrates a schematic representation of the TBD molecule. The protein is expressed as an N-terminal fusion protein with 6-Histidine tag, a seven amino acid rTEV protease cleavage site and the N-terminal fusion of the Target Binding Domain (TBD) of the xenotypic (murine) Mab raised against HBV sAg (Hybridoma 2C12). TBD is a fragment of the constant chain of the IgG1 Mab from 2C12 with the sequence of amino acids comprising the 8 amino acid peptide linker, five amino acids of the C.sub.H1 region, the hinge sequences, C.sub.H2 and C.sub.H3 region sequences (FIG. 5). The TBD fragment defined herein forms the parent molecule for the generation of fusion proteins with antigens derived from viruses or other infectious agents. FIG. 1A depicts the formation of dimeric TBD molecule formed via intermolecular disulphide bonds. FIG. 6 shows the nucleotide sequence of the Open Reading Frame (ORF) encoding the TBD protein and the deduced amino acid sequence as defined in FIG. 5.

Detail Description Paragraph - DETX (43):
[0129] FIG. 7 shows a schematic representation of chimeric antigen vaccine molecule, as produced in the insect cell expression system. This molecule is a fusion protein of N-terminal 6-His tag, rTEV protease cleavage site, HBV S1/S2 antigen, linker peptide, a part of the C.sub.H1 as well as C.sub.H2 and C.sub.H3 domains of the mouse monoclonal antibody from 2C12. Cleavage and purification will result in the generation of HBV S1/S2-TBD molecule. FIG. 8 shows the nucleotide and amino acid sequences of the chimeric antigen molecule. FIG. 9 shows the nucleotide and the deduced amino acid sequences of the expressed HBVS1/S2 protein.

Detail Description Paragraph - DETX (44):
[0130] FIG. 10 shows a schematic representation of the fusion protein of HBV S1/S2/S-TBD. FIG. 11 shows the nucleotide and deduced amino acid sequences of the ORF of the fusion protein. FIG. 12 shows the nucleotide and deduced amino acid sequences of the HBV S1/S2/S protein.

Detail Description Paragraph - DETX (45):
[0131] FIG. 13 illustrates the fusion protein of HBV core-TBD molecule as expressed in the insect cells. FIG. 14 shows the nucleotide and amino acid sequences in the ORF of the fusion protein. FIG. 15 shows the nucleotide and deduced amino acid sequences of the HBV Core protein.

Detail Description Paragraph - DETX (46):
[0132] Another embodiment of the present invention involves the production and use of fusion proteins generated from Duck Hepatitis B Virus (DHBV)

antigens and murine TBD. DHBV has been used as a very versatile animal model for the development of therapies for HBV, its human counterpart. DHBV genome encodes Surface antigen PreS/S, Core protein (Core) which form capsids and the polymerase enzyme which serves multiple functions.

Detail Description Paragraph - DETX (62):
[0145] The mouse IgG1 DNA sequences encoding amino acids of CH1-Hinge-CH2-CH3 region was generated form mRNA isolated from the hybridoma (2C12) which produces Mab against HBV surface antigen (sAg). Total mRNA was isolated using TRizol reagent (Gibco BRL cat. No. 15596-026) and the cDNA of the TBD was generated by RT-PCR using Superscript First-strand Synthesis (Invitrogen Cat. No. 11904-018). The PCR primers contained linker sequences encoding the linker peptide --SRPQGGGS-- at the 5' terminus, a unique Not I site at the 5' and a unique Hind III restriction site at the 3' end. The resulting cDNA contains (5' Not I)-linker sequence-CH1 (VDKKI)-CH2-CH3-(3' Hind III). Following digestion with the respective enzymes, the fragment is ligated with pFASTBACHTa expression vector plasmid (Invitrogen) using the same restriction enzyme sites. The 5' primer used for PCR amplification was (Sense) 5' TGTCATTCTGCGGCCGCAAGGCGGCGGGATCCGTGGACAAGAAAATTGTG CCAGG (Seq. ID No. 1) and the 3' primer was (antisense) 5' ACGAATCAAGCTTTGCAGCCCAGGAG- A (Seq. ID No. 2), which contained the Not I and Hind III sites, respectively. The following is the protocol used for directional cloning. The generated fragment was digested with the respective enzymes, purified on agarose gel and cloned into the vector plasmid. The DNA sequence and the correctness of the ORF were verified by standard sequencing methods.

Detail Description Paragraph - DETX (69):
Construction of HBV Surface Antigen S1/S2 and HBV S1/S2-TBD Fusion Protein Expression Vectors

Detail Description Paragraph - DETX (70):
[0151] The DNA encoding the HBV sAg fragment S1/S2 was generated from the plasmid pRSETB HBV S1/S2 template using PCR methodology. The primers used were: (sense) 5' GGATCCTGTACGATGACG (Seq. ID No. 5) and the 3' primer (antisense) 5' AGTCATTCTGCGGCCGCGAGTTCGTCACAGGGTCCCCGG (Seq. ID No. 6) containing the restriction enzyme site Not I. The 5' end contained a unique Bam H I site derived from the parent plasmid which was used for ligations. Amplified DNA was digested with Bam H I/Not I and ligated with pFastBacHTa expression vector to generate the expression plasmid for HBV S1/S2 protein. The fragment was ligated with the plasmid pFastBacHTa-TBD (described in example 1) following the digestion with the respective enzymes. This produced the expression plasmid pFastBacHTa HBV S1/S2-TBD. This plasmid was used to produce recombinant baculovirus (described in example 1) which expressed the chimeric antigen-TBD fusion protein: 6-His tag-rTEV protease cleavage site-HBVS1/S2-TBD (See FIGS. 7-9).

Detail Description Paragraph - DETX (72):
Construction of HBV Surface Antigen S1/S2/S and HBV S1/S2/S-TBD Fusion Protein Expression Vectors

Detail Description Paragraph - DETX (73):
[0152] The DNA encoding the HBV sAg fragment S1/S2/S was generated from the plasmid pALT HBV 991 (University of Alberta) template using PCR methodology. The 5' primer used for the PCR was (sense) 5' GATAAGGATCCTATGGGAGGTTGGTCATCAAAAC (Seq. ID No. 7), containing the restriction enzyme Nco I site. The PCR primer for 3' terminus was (antisense) 5' GTCATACTGCGGCCGCGAAATGTATACCCAGAGACAAAAG (Seq. ID No. 8), containing the restriction enzyme Not I site. Amplified cDNA was digested with the respective enzymes and ligated with pFastBacHTa expression vector to generate either the

expression plasmid for HBV S1/S2/S or the expression plasmid pFastBac HTa HBV S1/S2/S-TBD fusion protein (see FIGS. 10-11).

Detail Description Paragraph - DETX (75):
Construction of HBV Core Antigen and HBV Core-TBD Fusion Protein Expression Vectors

Detail Description Paragraph - DETX (76):
[0153] HBV produces the core proteins (Core) to encapsidate the replicating genome of the virus. There are two forms of the core one secreted into circulation, also known as the "e" antigen and the capsid forming core protein. The present invention also relates to the generation of expression plasmids to produce the Core protein as well as the core antigen-TBD fusion protein, in insect cells. The cDNA encoding the HBV Core protein was generated from the plasmid pALTHBV991 template using PCR technique. The 5' primer used for the PCR was (sense) 5' TGCGCTACCATGGACATTGACCCCTTATAAAG (Seq. ID No. 9) which contains the restriction enzyme Nco I site and the 3' primer used was (antisense) 5' TGTCATTCTGCGGCCGCGAACATTGAGATTCCCGAGATTGAG (Seq. ID No. 10), containing the restriction enzyme Not I site. The PCR-amplified cDNA was digested with the respective enzymes and ligated with pFastBacHTa expression vector to generate either the expression plasmid for HBV Core protein or the expression plasmid pFastBacHTa HBV Core-TBD fusion protein (see FIGS. 13-14).

Detail Description Paragraph - DETX (79):
[0154] DHBV has served as a powerful animal model in the development of antiviral therapy for HBV. Pekin ducks, congenitally infected with DHBV have been used to study-the mechanism of replication of the virus and for the screening of antiviral compounds. The present invention also describes the chimeric DHBV antigen-TBD molecules which could be used as therapeutic vaccines in DHBV-infected ducks, thus providing a viable animal model for the feasibility studies for a HBV therapeutic vaccines.

Detail Description Paragraph - DETX (88):
Chemically Cross-Linked HBV sAg-Fc (Murine)

Detail Description Paragraph - DETX (89):
[0158] HBV sAg was cross linked using the bifunctional cross linking agent DMS, a homobifunctional imidoester which react with amino groups on the proteins. The unreacted components were removed by gel filtration. The conjugate was characterized with respect to the stoichiometry of sAg/Fc in the conjugate and the fraction containing sAg:Fc at 1:1 ratio was chosen for antigen presentation assays using human monocyte-derived immature Dendritic cells (DCs). Immature DCs were cultured for four days with GM-CSF/IL4, incubated with the sAg-Fc conjugate and matured in the presence of TNF.alpha./IFN.alpha.. Autologous CD3+ T cells were added to the mature DCs. Following three rounds of exposure to the mature DCs, T cell stimulation was quantitated by measuring the production of intracellular IFN.gamma., using flow cytometry.

Detail Description Paragraph - DETX (92):
[0161] HBV sAg (US Biologicals; Cat# H 1910-27)

Detail Description Paragraph - DETX (112):
[0179] This protein has been cloned using the pFASTBAC HTa vector and the baculovirus system and expressed in Sf-9 and High Five insect cells, similar to the HBV fusion proteins. This was done as follows. The DNA encoding the HCV core fragment was generated from the plasmid pCV-H77c (NIH) template using PCR methodology.

Detail Description Paragraph - DETX (155):
[0206] To determine the extent of uptake of chimeric antigens (e.g. HBV S1/S2-TBD) compared with IgG1 and IgG2a, cells were incubated with various concentrations of the antigen, IgG1 (2C12, the parent Mab from which TBD was produced) and IgG2a (G155-178) for 1 hour at 37.degree. C. in AIM V media with 0.1% BSA. Cells were washed twice in PBSB and fixed with PBS with 2% PF overnight at 4.degree. C. Subsequently, the cells were washed twice in PBSB and permeablized with PBS containing 0.1% (w/v) saponin (Sigma) for 40 minutes at 20.degree. C.

Detail Description Paragraph - DETX (162):
[0211] The mouse IgG1 DNA sequences encoding amino acids of CH1-Hinge-CH2-CH3 region was generated form mRNA isolated from the hybridoma (2C12) which produces Mab against HBV surface antigen (sAg). Total mRNA was isolated using TRizol reagent (Gibco BRL cat. No. 15596-026) and the cDNA of the TBD was generated by RT-PCR using Superscript First-strand Synthesis (Invitrogen Cat. No. 11904-018). The PCR primers contained linker sequences encoding the linker peptide -SRPQGGGS- (Seq. ID No. _____) at the 5' terminus, a unique Not I site at the 5' and a unique Hind III restriction site at the 3' end. The resulting cDNA contains (5' Not I)-linker sequence-CH1 (VDKKI)-CH2-CH3-(3' Hind III). Following digestion with the respective enzymes, the fragment is ligated with pFASTBACHTa expression vector plasmid (Invitrogen) using the same restriction enzyme sites to generate-pFASTBACHTa-TBD. The 5' primer used for PCR amplification was (Sense) 5' TGTCATTCTGCGGCCGCAAGGCGGCGGGATCCGTGGACAAGAAAATTGTG CCAGG (Seq. ID No. 3) and the 3' primer was (antisense) 5' ACGAATCAAGCTTTGCAGCCCAGGAG- A (Seq. ID No. 4), which contained the Not I and Hind III sites, respectively. The following is the protocol used for directional cloning. The generated fragment was digested with the respective enzymes, purified on agarose gel and cloned into the vector plasmid. The DNA sequence and the correctness of the ORF were verified by standard sequencing methods. Nucleotide sequence of the ORF of TBD in the plasmid pFASTBACHTa-TBD and the deduced amino acid sequences of the expressed TBD protein from the ORF is shown in FIG. 32.

Detail Description Paragraph - DETX (168):
Construction of HBV Surface Antigen S1/S2 and HBV S1/S2-TBD Chimeric Fusion Protein Plasmids

Detail Description Paragraph - DETX (169):
[0214] The DNA encoding the HBV sAg fragment S1/S2 was generated from the plasmid pRSETB HBV S1/S2 template using PCR methodology. The primers used were: (sense) 5' GGATCCTGTACGATGACG (Seq. ID No. 5) and the 3' primer (antisense) 5' AGTCATTCTGCGGCCGCGAGTTCGTCACAGGGTCCCCGG (Seq. ID No. 6) containing the restriction enzyme site Not I. The 5' end contained a unique Bam H I site derived from the parent plasmid which was used for ligations. Amplified DNA was digested with Bam H I/Not I and ligated with pFastBacHTa expression vector to generate the expression plasmid for HBV S1/S2 protein. The fragment was ligated with the plasmid pFastBacHTa-TBD (described in example 3) following the digestion with the respective enzymes. This produced the expression plasmid pFastBacHTa HBV S1/S2-TBD. This plasmid was used to produce recombinant baculovirus (described in example 10) which expressed the chimeric antigen-TBD fusion protein: 6-His tag-rTEV protease cleavage site-HBVS1/S2-TBD. Nucleotide and deduced amino acid sequences from the ORFs of plasmid pFastBacHTa HBV S1/S2 are shown in FIG. 8. Nucleotide and deduced amino acid sequences from the ORFs of plasmid pFastBacHTa HBV S1/S2-TBD are shown in FIG. 33.

Detail Description Paragraph - DETX (171):
Expression and Purification of HBV Surface Antigen S1/S2 and HBVS1/S2-TBD

Chimeric Fusion Proteins

Detail Description Paragraph - DETX (172):
[0215] Recombinant bacmids of standardized multiplicity of infection (MOI) were used to infect High Five insect cells. For suspension cultures, cells were seeded at a density of 3.times.10.sup.5 cells/mL and incubated at 27.5.degree. C. with shaking at 138 rpm until the cell density reached 2-3.times.10.sup.6 cells/mL. Recombinant baculovirus was added to the cells. For the expression of the fusion protein HBV S1/S2-TBD, the MOI was 1 pfu/cell and for S1/S2, 2 pfu/cell were used. The incubation at 27.5.degree. C. was continued for 48 hrs. The cells were harvested by centrifugation at 2,500 rpm for 10 minutes at 4.degree. C. and used for the purification of the recombinant proteins.

Detail Description Paragraph - DETX (173):
[0216] HBV S1/S2 protein and the fusion protein HBV S1/S2-TBD fusion protein were expressed in Express Five Insect cells, purified as described in example 1.b. The proteins were subjected to electrophoresis on a 12% polyacrylamide gel and stained with coomassie blue.

Detail Description Paragraph - DETX (193):
Glycosylation of HBV S1/S2 Antigen Produced in Insect Cells Bind to DCs through CD206 Receptors.

Detail Description Paragraph - DETX (195):
[0226] HBV S1/S2, the antigen component of the chimeric antigen was expressed in both E. coli (no glycosylation) and in High Five insect cells (high mannose glycosylation). These antigens were compared for their binding to DCs. Glycosylated protein showed better binding and uptake by DCs (FIG. 40).

Detail Description Paragraph - DETX (204):
Glycosylation of HBV S1/S2 Antigen Imparts Immunogenicity to the Antigen and Generates Higher T Cell Responses

Detail Description Paragraph - DETX (205):
[0230] Glycosylation of HBV S1/S2 elicits increased immunogenicity and T Cell responses. The insect cell pathway of protein glycosylation is different from that of mammalian cells in that proteins synthesized in insect cells undergo glycosylation that results in high mannose content and a lack of terminal sialic acid residues in the secreted protein (Altman, Staudacher, et al 1999).

Detail Description Paragraph - DETX (206):
[0231] HBV S1/S2, the antigen component of the chimeric antigen was expressed in both E. coli (no glycosylation) and in High Five insect cells (high mannose glycosylation). These antigens were compared for T cell responses when presented by DCs. Both intracellular and secreted IFN.gamma. levels were measured and the results are presented in FIGS. 45 and 46.

Detail Description Paragraph - DETX (208):
Construction of HBV Core Antigen and HBV Core-TBD Fusion Protein Expression Vectors

Detail Description Paragraph - DETX (209):
[0232] HBV produces the core proteins (Core) to encapsidate the replicating genome of the virus. There are two forms of the core one secreted into circulation, also known as the "e" antigen and the capsid forming core protein. The present invention also relates to the generation of expression plasmids to

produce the Core protein as well as the core antigen-TBD fusion protein, in insect cells similar to examples described in example 19. The DNA encoding the HBV Core protein was generated from the plasmid pALTHBV991 template using PCR technique. The 5' primer used for the PCR was (sense) 5' TGCGCTACCATGGACATTGACCCCTTATAAA- G (Seq. ID No. 9) which contains the restriction enzyme Nco I site and the 3' primer used was (antisense) 5' TGTCATTCTGCGGCCGCGAACATTGAGATTCCCGAGATT- GAG (Seq. ID No. 8), containing the restriction enzyme Not I site. The PCR-amplified cDNA was digested with the respective enzymes and ligated with pFastBacHTa expression vector to generate either the expression plasmid for HBV Core protein or the expression plasmid pFastBacHTa HBV Core-TBD fusion protein. Nucleotide and deduced amino acid sequences from the ORFs of plasmid pFastBacHTa HBV core are shown in FIG. 15. Nucleotide and deduced amino acid sequences from the ORFs of plasmid pFastBacHTa HBV Core-TBD are shown in FIG. 14.

Detail Description Paragraph - DETX (212):
[0233] DHBV has served as a powerful animal model in the development of antiviral therapy for HBV. Pekin ducks, congenitally infected with DHBV have been used to study the mechanism of replication of the virus and for the screening of antiviral compounds. The present invention also describes the chimeric DHBV antigen-TBD molecules which could be used as therapeutic vaccines in DHBV-infected ducks, thus providing a viable animal model for the feasibility studies for a HBV therapeutic vaccines.

Detail Description Paragraph - DETX (253):
[0258] 7. Chimeric antigens include fusion proteins from HBV surface antigens (S1/S2), HBV Core and TBD, derived from the murine Mab 2C12.

Detail Description Paragraph - DETX (258):
[0263] 12. Chimeric antigen fusion protein HBV surface antigen S1/S2-TBD and HBV surface antigen S1/S2 has been purified.

Detail Description Paragraph - DETX (263):
[0268] 17. Chimeric antigen fusion protein HBV surface antigen S1/S2-TBD enhances the antigen presentation by professional antigen presenting cells (DCs).

Detail Description Paragraph - DETX (264):
[0269] 18. DCs loaded with the Chimeric antigen fusion protein HBV surface antigen S1/S2-TBD, on presentation to T cells elicit an immune response.

Detail Description Paragraph - DETX (286):
[0291] Description of Artificial Sequence: Seq. ID No. 5: HBV S1/S2 5' PCR amplification primer

Detail Description Paragraph - DETX (291):
[0296] Description of Artificial Sequence: Seq. ID No.6: HBV S1/S2 3' PCR amplification primer

Detail Description Paragraph - DETX (296):
[0301] Description of Artificial Sequence: Seq. ID No. 7: HBV S1/S2/S 5' PCR Amplification Primer

Detail Description Paragraph - DETX (301):
[0306] Description of Artificial Sequence: Seq. ID No. 8: HBV S1/S2/S 3' PCR Amplification Primer

Claims Text - CLTX (3):
2. The composition of claim 1 wherein the immune response domain comprises

one or more proteins and recombinant proteins selected from the group consisting of one or more HBV proteins and recombinant proteins thereof, and one or more DHBV proteins and recombinant proteins thereof, and one or more HCV proteins and recombinant proteins thereof.

Claims Text - CLTX (4):
3. The composition of claim 2 wherein the immune response domain comprises one or more of HBV proteins and recombinant proteins selected from a group consisting of HBV S1/S2, HBV S1/S2/S, HBV Core, HBV Core ctm and HBV polymerase.

Claims Text - CLTX (21):
20. A composition for eliciting a T-cell response in vivo comprising contacting an antigen presenting cell with a chimeric antigen comprising an immune response domain and a target binding domain, wherein the immune response domain comprises one or more sequences comprising an HBV core protein, HBV S protein, HBV S1 protein, HBV S2 protein, combinations thereof, and recombinant molecules thereof, and wherein the target binding domain comprises a xenotypic antibody fragment.

PGPUB-DOCUMENT-NUMBER: 20030207873

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030207873 A1

TITLE: Inhibitors of Src and other protein kinases

PUBLICATION-DATE: November 6, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 119890

DATE FILED: April 10, 2002

US-CL-CURRENT: 514/227.8, 514/235.8 , 514/241 , 514/252.19 , 514/275 , 544/122 , 544/212 , 544/295 , 544/331 , 544/60

ABSTRACT:

The present invention provides compounds of formula I: 1
wherein A is N or CR, and R.sup.1, G, and R.sup.2, are as described in the specification. These compounds are inhibitors of protein kinase, particularly inhibitors of Src mammalian protein kinase involved in cell proliferation, cell death and response to extracellular stimuli. The invention also relates to methods for producing these inhibitors. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Patent Application 60/282,935 filed Apr. 10, 2001, the contents of which are incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (9):
[0008] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J., 18, 5019, (1999) and Klein et al., Mol. Cell. Biol., 17, 6427 (1997).

Summary of Invention Paragraph - BSTX (22):
[0021] Accordingly, there is still a great need to develop potent inhibitors of JNK3, Src, and Lck protein kinases that are useful in treating various diseases or conditions associated with JNK3, Src, and Lck activation.

PGPUB-DOCUMENT-NUMBER: 20030198975

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030198975 A1

TITLE: Proteins associated with cell growth, differentiation, and death

PUBLICATION-DATE: October 23, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 287218

DATE FILED: October 31, 2002

RELATED-US-APPL-DATA:

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parent continuation-of PCT/US02/11152 20020405 US PENDING

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non-provisional-of-provisional 60295340 20010601 US

non-provisional-of-provisional 60293727 20010525 US

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non-provisional-of-provisional 60287228 20010427 US

non-provisional-of-provisional 60286820 20010426 US

non-provisional-of-provisional 60283294 20010411 US

non-provisional-of-provisional 60282110 20010406 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
WO	PCT/US02/11152	2002WO-PCT/US02/11152	April 5, 2002

US-CL-CURRENT: 435/6, 435/183 , 435/320.1 , 435/325 , 435/69.1 , 514/44 , 530/350 , 530/388.26 , 536/23.2

ABSTRACT:

The invention provides human proteins associated with cell growth, differentiation, and death (CGDD) and polynucleotides which identify and encode CGDD. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of CGDD.

[0001] This application is a continuation application of PCT application PCT/US02/11152, filed in English on Apr. 5, 2002 and which will be published in English, which claims the benefit of provisional applications U.S. Ser. No. 60/282,110, filed Apr. 6, 2001, U.S. Ser. No. 60/283,294, filed Apr. 11, 2001, U.S. Ser. No. 60/286,820, filed Apr. 26, 2001, U.S. Ser. No. 60/287,228, filed Apr. 27, 2001, U.S. Ser. No. 60/291,662, filed May 16, 2001, U.S. Ser. No. 60/291,846, filed May 18, 2001, U.S. Ser. No. 60/293,727, filed May 25, 2001, U.S. Ser. No. 60/295,340, filed Jun. 1, 2001, U.S. Ser. No. 60/295,263, filed Jun. 1, 2001, and U.S. Ser. No. 60/349,705, filed Jan. 15, 2002, all of which applications and patents are hereby incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (120):

[0119] Ras regulates other signaling pathways by direct interaction with different cellular targets (Katz, M. E. and McCormick, F. (1997) Curr. Opin. Genet. Dev. 7:75-79). One such target is RalGDS, a guanine nucleotide dissociation stimulator for the Ras-like GTPase, Ral (Albright, C. F. et al. (1993) EMBO J. 12:339-347). RalGDS couples the Ras and Ral signaling pathways. Epidermal growth factor (EGF) stimulates the association of RalGDS with Ras in mammalian cells, which activates the GEF activity of RalGDS (Kikuchi, A. and Williams, L. T. (1996) J. Biol. Chem. 271:588-594; Urano, T. et al. (1996) EMBO J. 15:810-816). Ral activation by Ral-GDS leads to activation of Src, a

tyrosine kinase that phosphorylates other molecules including transcription factors and components of the actin cytoskeleton (Goi, T. et al. (2000) EMBO J. 19:623-630). Ral interacts with a number of signaling molecules including Ral-binding protein, a GAP for the Rho-like GTPases; Cdc42 and Rac, which regulate cytoskeletal rearrangement; and phospholipase D1, which is involved in vesicular trafficking (Feig, L. A. et al. (1996) Trends Biochem. Sci. 21:438-441; Voss, M. et al. (1999) J. Biol. Chem. 274:34691-34698).

Summary of Invention Paragraph - BSTX (359):

[0355] In another embodiment of the invention, polynucleotides encoding CGDD may be used for somatic or germline gene therapy. Gene therapy may be performed to (i) correct a genetic deficiency (e.g., in the cases of severe combined immunodeficiency (SCID)-X1 disease characterized by X-linked inheritance (Cavazzana-Calvo, M. et al. (2000) Science 288:669-672), severe combined immunodeficiency syndrome associated with an inherited adenosine deaminase (ADA) deficiency (Blaese, R. M. et al. (1995) Science 270:475-480; Bordignon, C. et al. (1995) Science 270:470-475), cystic fibrosis (Zabner, J. et al. (1993) Cell 75:207-216; Crystal, R. G. et al. (1995) Hum. Gene Therapy 6:643-666; Crystal, R. G. et al. (1995) Hum. Gene Therapy 6:667-703), thalassamias, familial hypercholesterolemia, and hemophilia resulting from Factor VIII or Factor IX deficiencies (Crystal, R. G. (1995) Science 270:404-410; Verma, I. M. and N. Somia (1997) Nature 389:239-242)), (ii) express a conditionally lethal gene product (e.g., in the case of cancers which result from unregulated cell proliferation), or (iii) express a protein which affords protection against intracellular parasites (e.g., against human retroviruses, such as human immunodeficiency virus (HIV) (Baltimore, D. (1988) Nature 335:395-396; Poeschla, E. et al. (1996) Proc. Natl. Acad. Sci. USA 93:11395-11399), hepatitis B or C virus (HBV, HCV); fungal parasites, such as Candida albicans and Paracoccidioides brasiliensis; and protozoan parasites such as Plasmodium falciparum and Trypanosoma cruzi). In the case where a genetic deficiency in CGDD expression or regulation causes disease, the expression of CGDD from an appropriate population of transduced cells may alleviate the clinical manifestations caused by the genetic deficiency.

PGPUB-DOCUMENT-NUMBER: 20030144309

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030144309 A1

TITLE: Inhibitors of Src and other protein kinases

PUBLICATION-DATE: July 31, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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APPL-NO: 10/ 146984

DATE FILED: May 16, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60291340 20010516 US

US-CL-CURRENT: 514/275, 514/227.8 , 514/235.8 , 514/252.19 , 514/253.09 , 514/341 , 544/122 , 544/124 , 544/295 , 544/331 , 544/360 , 544/60 , 546/275.4

ABSTRACT:

The present invention provides compounds of formula I: 1 wherein A is N or CR, and G, R.sup.1, R.sup.2 and R.sup.3 are as described in the specification. These compounds are inhibitors of protein kinase, particularly inhibitors of Src mammalian protein kinase involved in cell proliferation, cell death and response to extracellular stimuli. The invention also relates to methods for producing these inhibitors. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application 60/291,340 filed May 16, 2001, the contents of which are incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (9):
[0008] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J., 18, 5019, (1999) and Klein et al., Mol.Cell. Biol., 17, 6427 (1997).

Summary of Invention Paragraph - BSTX (22):
[0021] Accordingly, there is still a great need to develop potent inhibitors of JNK3, Src, and Lck protein kinases that are useful in treating various diseases or conditions associated with JNK3, Src, and Lck activation.

US-PAT-NO: 6642227

DOCUMENT-IDENTIFIER: US 6642227 B2

TITLE: Inhibitors of c-Jun N-terminal kinases (JNK) and other protein kinases

DATE-ISSUED: November 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cao; Jingrong	Newton	MA	N/A	N/A
Green; Jeremy	Burlington	MA	N/A	N/A
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Wang; Jian	Boston	MA	N/A	N/A
Ledeboer; Mark	Acton	MA	N/A	N/A
Harrington; Edmund	South Boston	MA	N/A	N/A
Gao; Huai	Natick	MA	N/A	N/A

APPL-NO: 10/ 121035

DATE FILED: April 10, 2002

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims benefit of priority to U.S. provisional applications 60/283,621 filed Apr. 13, 2001, 60/329,440 filed Oct. 15, 2001 and 60/292,974 filed May 23, 2001.

US-CL-CURRENT: 514/227.8, 514/235.8 , 514/252.19 , 514/275 , 544/122 , 544/331 , 544/60

ABSTRACT:

The present invention provides compounds of formula I: ##STR1##

or a pharmaceutically acceptable derivative o thereof, wherein A, B, R.sup.a, R.sup.1, R.sup.2, R.sup.3, and R.sup.4 are as described in the specification. These compounds are inhibitors of protein kinase, particularly inhibitors of JNK, a mammalian protein kinase involved cell proliferation, cell death and response to extracellular stimuli; Lck and Src kinase. The invention also relates to methods for producing these inhibitors. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

31 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (19):
Src also plays a role in the replication of hepatitis B virus. The virally

encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J., 18, 5019, (1999) and Klein et al., Mol. Cell. Biol., 17, 6427 (1997).

Brief Summary Text - BSTX (22):

Accordingly, there is still a great need to develop potent inhibitors of JNKs and Src family kinases that are useful in treating various conditions associated with JNK and Src activation.

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	174	hbx	US-PGPUB; USPAT	OR	OFF	2004/09/21 09:05
L2	492	(hepatitis adj b adj virus or hbv) near4 inhibit$8	US-PGPUB; USPAT	OR	OFF	2004/09/21 09:07
L3	26	1 and 2	US-PGPUB; USPAT	OR	OFF	2004/09/21 09:07
L4	11	hbx near4 inhibit$8	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:02
L5	10160	src	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:08
L6	794441	activat$8	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:08
L7	239593	upstream	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:09
L8	3912	6 near5 7	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:09
L9	27	8 same 5	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:13
L10	97	5 same 6 same 7	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:13
L11	334	5 near2 (activator$1 or activation)	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:20
L12	4205	hbv or hbx	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:20
L13	19	11 and 12	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:26
L14	798	5 near5 6	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:27
L15	55	12 and 14	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:27

PGPUB-DOCUMENT-NUMBER: 20040132043

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040132043 A1

TITLE: Proteins Associated with cell growth, differentiation, and death

PUBLICATION-DATE: July 8, 2004

INVENTOR-INFORMATION:

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APPL-NO: PCT/US02/11152
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US-CL-CURRENT: 435/6, 435/320.1 , 435/325 , 435/69.1 , 530/350 , 536/23.5

ABSTRACT:

The invention provides human proteins associated with cell growth, differentiation, and death (CGDD) and polynucleotides which identify and encode CGDD. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of CGDD.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (120):
[0118] Ras regulates other signaling pathways by direct interaction with different cellular targets (Katz, M. E. and McCormick, F. (1997) Curr. Opin. Genet. Dev. 7:75-79). One such target is RalGDS, a guanine nucleotide dissociation stimulator for the Ras-like GTPase, Ral (Albright, C. F. et al. (1993) EMBO J. 12:339-347). RalGDS couples the Ras and Ral signaling pathways. Epidermal growth factor (EGF) stimulates the association of RalGDS with Ras in mammalian cells, which activates the GEF activity of RalGDS (Kikuchi, A. and Williams, L. T. (1996) J. Biol. Chem. 271:588-594; Urano, T. et al. (1996) EMBO J. 15:810-816). Ral activation by Ral-GDS leads to activation of Src, a tyrosine kinase that phosphorylates other molecules including transcription factors and components of the actin cytoskeleton (Goi, T. et al. (2000) EMBO J. 19:623-630). Ral interacts with a number of signaling molecules including Ral-binding protein, a GAP for the Rho-like GTPases; Cdc42 and Rac, which regulate cytoskeletal rearrangement; and phospholipase D1, which is involved in vesicular trafficking (Feig, L. A. et al. (1996) Trends Biochem. Sci. 21:438-441; Voss, M. et al. (1999) J. Biol. Chem. 274:34691-34698).

Detail Description Paragraph - DETX (175):
[0352] In another embodiment of the invention, polynucleotides encoding CGDD may be used for somatic or germline gene therapy. Gene therapy may be performed to (i) correct a genetic deficiency (e.g., in the cases of severe combined immunodeficiency (SCID)-X1 disease characterized by X-linked inheritance (Cavazzana-Calvo, M. et al. (2000) Science 288:669-672), severe combined immunodeficiency syndrome associated with an inherited adenosine deaminase (ADA) deficiency (Blaese, R. M. et al. (1995) Science 270:475-480; Bordignon, C. et al. (1995) Science 270:470-475), cystic fibrosis (Zabner, J. et al. (1993) Cell 75:207-216; Crystal, R. G. et al. (1995) Hum. Gene Therapy 6:643-666; Crystal, R. G. et al. (1995) Hum. Gene Therapy 6:667-703), thalassamias, familial hypercholesterolemia, and hemophilia resulting from Factor VIII or Factor IX deficiencies (Crystal, R. G. (1995) Science 270:404-410; Verma, I. M. and N. Somia (1997) Nature 389:239-242)), (ii) express a conditionally lethal gene product (e.g., in the case of cancers which result from unregulated cell proliferation), or (iii) express a protein which affords protection against intracellular parasites (e.g., against human retroviruses, such as human immunodeficiency virus (HIV) (Baltimore, D. (1988) Nature 335:395-396; Poeschla, E. et al. (1996) Proc. Natl. Acad. Sci. USA 93:11395-11399), hepatitis B or C virus (HBV, HCV); fungal parasites, such as Candida albicans and Paracoccidioides brasiliensis; and protozoan parasites such as Plasmodium falciparum and Trypanosoma cruzi). In the case where a genetic deficiency in CGDD expression or regulation causes disease, the expression of CGDD from an appropriate population of transduced cells may alleviate the clinical manifestations caused by the genetic deficiency.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040115629 A1

TITLE: Molecules for diagnostics and therapeutics

PUBLICATION-DATE: June 17, 2004

INVENTOR-INFORMATION:

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DATE FILED: July 9, 2003

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APPL-NO: PCT/US02/01009
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371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/6, 435/320.1 , 435/325 , 435/69.1 , 530/350 , 536/23.5

ABSTRACT:

The present invention provides purified human polynucleotides for diagnostics and therapeutics (dithp). Also en-compassed are the polypeptides (DITHP) encoded by dithp. The invention also provides for the use of dithp, or complements, oligonucleotides, or fragments thereof in diagnostic assays. The invention further provides for vectors and host cells containing dithp for the expression of DITHP. The invention additionally provides for the use of isolated and purified DITHP to induce antibodies and to screen libraries of compounds and the use of anti-DITHP antibodies in diagnostic assays. Also provided are microarrays containing dithp and methods of use.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (289):

[0287] Many growth factor receptors, including receptors for epidermal growth factor, platelet-derived growth factor, fibroblast growth factor, as well as the growth modulator .alpha.-thrombin, contain intrinsic protein kinase activities. When growth factor binds to the receptor, it triggers the autophosphorylation of a serine, threonine, or tyrosine residue on the receptor. These phosphorylated sites are recognition sites for the binding of other cytoplasmic signaling proteins. These proteins participate in signaling pathways that eventually link the initial receptor activation at the cell surface to the activation of a specific intracellular target molecule. In the case of tyrosine residue autophosphorylation, these signaling proteins contain a common domain referred to as a Src homology (SH) domain. SH2 domains and SH3 domains are found in phospholipase C-.gamma., PI-3-K p85 regulatory subunit, Ras-GTPase activating protein, and pp60.sup.c-Src (Lowenstein, E. J. et al. (1992) Cell 70:431-442). The cytokine family of receptors share a different common binding domain and include transmembrane receptors for growth hormone (GH), interleukins, erythropoietin, and prolactin.

Detail Description Paragraph - DETX (193):

[0770] The dithp of the invention may be used for somatic or germline gene therapy. Gene therapy may be performed to (i) correct a genetic deficiency (e.g., in the cases of severe combined immunodeficiency (SCID)-X1 disease characterized by X-linked inheritance (Cavazzana-Calvo, M. et al. (2000) Science 288:669-672), severe combined immunodeficiency syndrome associated with an inherited adenosine deaminase (ADA) deficiency (Blaese, R. M. et al. (1995) Science 270:475-480; Bordignon, C. et al. (1995) Science 270:470-475), cystic fibrosis (Zabner, J. et al. (1993) Cell 75:207-216; Crystal, R. G. et al. (1995) Hum. Gene Therapy 6:643-666; Crystal, R. G. et al. (1995) Hum. Gene Therapy 6:667-703), thalassemias, familial hypercholesterolemia, and hemophilia resulting from Factor VIII or Factor IX deficiencies (Crystal, R. G. (1995) Science 270:404-410; Verma, I. M. and Somia, N. (1997) Nature 389:239-242)), (ii) express a conditionally lethal gene product (e.g., in the case of cancers which result from unregulated cell proliferation), or (iii) express a protein which affords protection against intracellular parasites (e.g., against human retroviruses, such as human immunodeficiency virus (HIV) (Baltimore, D. (1988) Nature 335:395-396; Poeschla, E. et al. (1996) Proc. Natl. Acad. Sci. USA. 93:11395-11399), hepatitis B or C virus (HBV, HCV); fungal parasites, such as Candida albicans and Paracoccidioides brasiliensis; and protozoan parasites such as Plasmodium falciparum and Trypanosoma cruzi). In the case where a genetic deficiency in dithp expression or regulation causes disease, the expression of dithp from an appropriate population of transduced cells may alleviate the clinical manifestations caused by the genetic deficiency.

PGPUB-DOCUMENT-NUMBER: 20040110177

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040110177 A1

TITLE: Method for identifying functional nucleic acids

PUBLICATION-DATE: June 10, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 470845

DATE FILED: July 31, 2003

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APPL-NO: PCT/EP02/01073
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PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/6

ABSTRACT:

The present invention relates to a method for identifying nucleic acid molecules functionally associated with a desired phenotype.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (22):
[0022] Alternatively, the desired phenotype may be selected from other properties such as production of secreted protein, e.g. insulin, growth hormone, interferons etc., susceptibility or resistance to pathogens, e.g. viruses such as HCV, HBV or other pathogens, senescence and regulation of cell functions, i.e. the identification of genes that regulate certain cell functions e.g. identification of negative regulators of insulin receptor activity comprising a screen for cell clones with upregulated insulin receptor activity.

Detail Description Paragraph - DETX (150):
[0187] 76. Nada, S., et al., Constitutive activation of Src family kinases in mouse embryos that lack Csk Cell, 1993. 73(6): p. 1125-35.

PGPUB-DOCUMENT-NUMBER: 20040106615

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040106615 A1

TITLE: Protein kinase inhibitors and uses thereof

PUBLICATION-DATE: June 3, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 639784

DATE FILED: August 12, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60403256 20020814 US

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US-CL-CURRENT: 514/242, 514/247 , 514/252.03 , 514/275 , 544/183 , 544/238 , 544/331

ABSTRACT:

Described herein are compounds that are useful as protein kinase inhibitors having the formulae I and V: 1
or a pharmaceutically acceptable salt thereof, wherein Ring B, Z.sup.1, Z.sup.2, U, T, m, n, p, Q, Q', R.sup.1, R.sup.2, R.sup.x, R.sup.3, and R.sup.6 are as defined herein. These compounds, and pharmaceutically acceptable compositions thereof, are useful for treating or lessening the severity of a variety of disorders, including stroke, inflammatory disorders, autoimmune diseases such as SLE lupus and psoriasis, proliferative disorders such as cancer, and conditions associated with organ transplantation.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Applications 60/403,256 filed Aug. 14, 2002 and 60/416,802 filed Oct. 8, 2002, the contents of which are incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (79):
[0078] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J. 1999, 18, 5019, and Klein et al., Mol. Cell. Biol. 1997, 17, 6427.

PGPUB-DOCUMENT-NUMBER: 20040106125

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040106125 A1

TITLE: Neurotransmission-associated proteins

PUBLICATION-DATE: June 3, 2004

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APPL-NO: 10/ 468334

DATE FILED: August 15, 2003

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US-CL-CURRENT: 435/6, 424/143.1 , 435/320.1 , 435/325 , 435/69.1 , 530/350 , 530/388.22

ABSTRACT:

The invention provides human neurotransmission-associated proteins (NTRAN) and polynucleotides which identify and encode NTRAN. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of NTRAN.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (89):
[0087] Many growth factor receptors, including receptors for epidermal

growth factor, platelet-derived growth factor, fibroblast growth factor, as well as the growth modulator x-thrombin, contain intrinsic protein kinase activities. When growth factor binds to the receptor, it triggers the autophosphorylation of a serine, threonine, or tyrosine residue on the receptor. These phosphorylated sites are recognition sites for the binding of other cytoplasmic signaling proteins. These proteins participate in signaling pathways that eventually link the initial receptor activation at the cell surface to the activation of a specific intracellular target molecule. In the case of tyrosine residue autophosphorylation, these signaling proteins contain a common domain referred to as a Src homology (SH) domain. SH2 domains and SH3 domains are found in phospholipase C-.gamma., PI-3-K p85 regulatory subunit, Ras-GTPase activating protein, and pp60.sup.c-src (Lowenstein, E. J. et al. (1992) Cell 70:431-442). The cytokine family of receptors share a different common binding domain and include transmembrane receptors for growth hormone (GH), interleukins, erythropoietin, and prolactin.

Detail Description Paragraph - DETX (171):

[0325] In another embodiment of the invention, polynucleotides encoding NTRAN may be used for somatic or germline gene therapy. Gene therapy may be performed to (i) correct a genetic deficiency (e.g., in the cases of severe combined immunodeficiency (SCID)-X1 disease characterized by X-linked inheritance (Cavazzana-Calvo, M. et al. (2000) Science 288:669-672), severe combined immunodeficiency syndrome associated with an inherited adenosine deaminase (ADA) deficiency (Blaese, R. M. et al. (1995) Science 270:475-480; Bordignon, C. et al. (1995) Science 270:470-475), cystic fibrosis (Zabner, J. et al. (1993) Cell 75:207-216; Crystal, R. G. et al. (1995) Hum. Gene Therapy 6:643-666; Crystal, R. G. et al. (1995) Hum. Gene Therapy 6:667-703), thalassamias, familial hypercholesterolemia, and hemophilia resulting from Factor VIII or Factor IX deficiencies (Crystal, R. G. (1995) Science 270:404-410; Verma, I. M. and N. Somia (1997) Nature 389.239-242)), (ii) express a conditionally lethal gene product (e.g., in the case of cancers which result from unregulated cell proliferation), or (iii) express a protein which affords protection against intracellular parasites (e.g., against human retroviruses, such as human immunodeficiency virus (HIV) (Baltimore, D. (1988) Nature 335:395-396; Poeschla, E. et al. (1996) Proc. Natl. Acad. Sci. USA 93:11395-11399), hepatitis B or C virus (HBV, HCV); fungal parasites, such as Candida albicans and Paracoccidioides brasiliensis; and protozoan parasites such as Plasmodium falciparum and Trypanosoma cruzi). In the case where a genetic deficiency in NTRAN expression or regulation causes disease, the expression of NTRAN from an appropriate population of transduced cells may alleviate the clinical manifestations caused by the genetic deficiency.

PGPUB-DOCUMENT-NUMBER: 20040097707

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040097707 A1

TITLE: Receptors and membrane-associated proteins

PUBLICATION-DATE: May 20, 2004

INVENTOR-INFORMATION:

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Tang, Y. Tom	San Jose	CA	US	
Yue, Henry	Sunnyvale	CA	US	
Thangavelu, Kavitha	Mountain View	CA	US	
Xu, Yuming	Mountain View	CA	US	
Arvizu, Chandra S.	Menlo Park	CA	US	
Warren, Bridget A.	Cupertino	CA	US	
Yao, Monique G.	Carmel	IN	US	
Au-Young, Janice K.	Brisbane	CA	US	
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DATE FILED: July 18, 2003

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DATE-FILED: Jan 16, 2002
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US-CL-CURRENT: 530/350, 435/320.1 , 435/325 , 435/6 , 435/69.1 , 530/388.22 , 536/23.5

ABSTRACT:

The invention provides human receptors and membrane-associated proteins (REMAP) and polynucleotides which identify and encode REMAP. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists.

The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of REMAP.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (16):

[0014] Many growth factor receptors, including receptors for epidermal growth factor, platelet-derived growth factor, fibroblast growth factor, as well as the growth modulator .alpha.-thrombin, contain intrinsic protein kinase activities. When growth factor binds to the receptor, it triggers the autophosphorylation of a serine, threonine, or tyrosine residue on the receptor. These phosphorylated sites are recognition sites for the binding of other cytoplasmic signaling proteins. These proteins participate in signaling pathways that eventually link the initial receptor activation at the cell surface to the activation of a specific intracellular target molecule. In the case of tyrosine residue autophosphorylation, these signaling proteins contain a common domain referred to as a Src homology (SH) domain. SH2 domains and SH3 domains are found in phospholipase C-.gamma., PI-3-K p85 regulatory subunit, Ras-GTPase activating protein, and pp60.sub.c-src (Lowenstein, E. J. et al. (1992) Cell 70:431-442). The cytokine family of receptors share a different common binding domain and include transmembrane receptors for growth hormone (GH), interleukins, erythropoietin, and prolactin.

Summary of Invention Paragraph - BSTX (310):

[0304] In another embodiment of the invention, polynucleotides encoding REMAP may be used for somatic or germline gene therapy. Gene therapy may be performed to (i) correct a genetic deficiency (e.g., in the cases of severe combined immunodeficiency (SCID)-X1 disease characterized by X-linked inheritance (Cavazzana-Calvo, M. et al. (2000) Science 288:669-672), severe combined immunodeficiency syndrome associated with an inherited adenosine deaminase (ADA) deficiency (Blaese, R. M. et al. (1995) Science 270:475-480; Bordignon, C. et al. (1995) Science 270:470-475), cystic fibrosis (Zabner, J. et al. (1993) Cell 75:207-216; Crystal, R. G. et al. (1995) Hum Gene Therapy 6:643-666; Crystal, R. G. et al. (1995) Hum. Gene Therapy 6:667-703), thalassamias, familial hypercholesterolemia, and hemophilia resulting from Factor VIII or Factor IX deficiencies (Crystal, R. G. (1995) Science 270:404-410; Verma, I. M. and N. Somia (1997) Nature 389:239-242)), (ii) express a conditionally lethal gene product (e.g., in the case of cancers which result from unregulated cell proliferation), or (iii) express a protein which affords protection against intracellular parasites (e.g., against human retroviruses, such as human immunodeficiency virus (HIV) (Baltimore, D. (1988) Nature 335:395-396; Poeschla, E. et al. (1996) Proc. Natl. Acad. Sci. USA 93:11395-11399), hepatitis B or C virus (HBV, HCV); fungal parasites, such as Candida albicans and Paracoccidioides brasiliensis; and protozoan parasites such as Plasmodium falciparum and Trypanosoma cruzi). In the case where a genetic deficiency in REMAP expression or regulation causes disease, the expression of REMAP from an appropriate population of transduced cells may alleviate the clinical manifestations caused by the genetic deficiency.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040097531 A1

TITLE: Inhibitors of c-Jun N-terminal kinases (JNK) and other protein kinases

PUBLICATION-DATE: May 20, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 616560

DATE FILED: July 9, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60395202 20020709 US

US-CL-CURRENT: 514/275, 514/341 , 514/342 , 544/331 , 546/270.4 , 546/271.4 , 546/272.7

ABSTRACT:

The present invention provides compounds of formula I: 1 or a pharmaceutically acceptable derivative thereof, wherein R.sup.1, R.sup.2, A, G, and W are as described in the specification. These compounds are inhibitors of protein kinase, particularly inhibitors of JNK, a mammalian protein kinase involved cell proliferation, cell death and response to extracellular stimuli, Lck, Src, and Aurora kinases. The invention also relates to methods for producing these inhibitors. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application 60/395,202, filed Jul. 9, 2002, which is hereby incorporated by reference in its entirety.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (19):
[0018] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus [Klein et al., EMBO J., 18:5019, (1999) and Klein et al., Mol. Cell. Biol., 17:6427 (1997)].

Summary of Invention Paragraph - BSTX (31):
[0030] There is a continued need to develop potent inhibitors of JNKs, Src

family kinases, and Aurora family kinases that are useful in treating or preventing various conditions associated with JNK, Src, and Aurora activation.

Summary of Invention Paragraph - BSTX (193):

[0190] The activity of the compounds of this invention as kinase inhibitors may be assayed in vitro, in vivo or in a cell line. In vitro assays include assays that determine inhibition of either the kinase activity or ATPase activity of activated enzyme, for example JNK, Lck, Src or Aurora-2. Alternate in vitro assays quantitate the ability of the inhibitor to bind to JNK, Lck, Src, or Aurora-2 and may be measured either by radiolabelling the inhibitor prior to binding, isolating the inhibitor/JNK, inhibitor/Lck, or inhibitor/Src complex and determining the amount of radiolabel bound, or by running a competition experiment where new compounds are incubated with JNK, Lck, Src, or Aurora-2 bound to known radioligands. One may use any type or isoform of JNK, Lck, Src, or Aurora-2, depending upon which JNK, Lck, Src, or Aurora-2 type or isoform is to be inhibited. The details of the conditions used for the enzymatic assays are set forth in the Examples hereinbelow.

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TITLE: Thienopyrimidine-based inhibitors of the src family

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INVENTOR-INFORMATION:

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ABSTRACT:

Various thienopyrimidine-based analog compounds are able to selectively inhibit the Src family of tyrosine kinases. These compounds are useful in the treatment of various diseases including hyperproliferative diseases, hematologic diseases, osteoporosis, neurological diseases, autoimmune diseases, allergic/immunological diseases, or viral infections.

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Summary of Invention Paragraph - BSTX (7):
[0007] Src activity is greatly increased in many human cancers: breast cancer (Ottenhoff-Kalff et al., 1992; Partanen, 1994), stomach cancer (Takeshima et al., 1991), colon cancer (Rosen et al., 1986; Bolen et al., 1985; Bolen et al., 1987; Cartwright et al., 1989; Cartwright et al., 1990; Talamonti et al., 1992; Talamonti et al., 1993; Termuhlen et al., 1993), hairy cell leukemia and a subgroup of B-cell lymphomas (Lynch et al., 1993), low grade human bladder carcinoma (Fanning et al., 1992), neuroblastoma (Bolen et al., 1985; O'Shaughnessy et al., 1987; Bjelfman et al., 1990), ovarian cancer (Wiener et al., 1999) and non-small cell lung carcinoma (Budde et al., 1994). In the case of colon cancer, Src is activated more frequently than Ras or p53 (Jessup and Gallick, 1993), and undergoes two distinct activations

corresponding with malignant transformation of colonocytes (Cartwright et al., 1990) and tumor progression (Talamonti et al., 1991, 1992; Termuhlen et al., 1993). Antisense to Src inhibits growth of human monoblastoid leukemia cells (Waki et al., 1994), K562 human leukemia cells (Kitanaka et al., 1994) and HT-29 human colon cancer cells (Staley et al., 1995). Src activity was reduced in a human ovarian cancer cell line (SKOv-3) by antisense technology. The reduced Src activity in SKOv-3 was associated with altered cellular morphology, reduced anchorage-independent growth, diminished tumor growth and reduced vascular endothelial growth factor mRNA expression in vitro (Wiener et al., 1999). In addition, growth inhibition of colon tumor (Garcia et al., 1991; Novotny-Smith & Gallick, 1992) and neuroblastoma cell lines (Preis et al., 1988) correlate with decreases in tyrosine kinase activity of Src. In a colon adenocarcinoma cell line, HT29, the mRNA expression of vascular endothelial growth factor (VEGF) was decreased in proportion to the decrease in Src kinase activity caused by expression of a Src antisense expression vector. In nude mice, there was a decrease in tumor vascularity in subcutaneous tumors from Src antisense transfectants (Ellis et al., 1998).

Summary of Invention Paragraph - BSTX (12):
[0012] Herpesviridae, papovaviridae, and retroviridae have been shown to interact with non-receptor tyrosine kinases and use them as signaling intermediates. The HIV-1 Nef protein interacts with members of the Src family of tyrosine kinases. Nef mediates downregulation of CD4 membrane expression, modification of T-cell activation pathways, and increases virus infectivity (Collette et al., 1997). The HBx protein of the hepatitis B virus is essential for infection by hepadnaviruses and activates Ras by activating the Src family of tyrosine kinases. The activation of Ras is necessary for the ability of the HBx protein to stimulate transcription and release growth arrest in quiescent cells (Klein and Schneider, 1997). Activity of the Src family of tyrosine kinases is altered by association with viral proteins such as mouse and hamster polyomavirus middle-T antigens, Epstein-Barr virus LMP2A, and herpesvirus saimiri Tip (Dunant and Ballmer-Hofer, 1997).

Detail Description Paragraph - DETX (218):
[0282] Bolen J B, Veillette A, Schwartz A M, Deseau V, Rosen N: Activation of pp60c-src in human colon carcinoma and normal human colon mucosal cells. Oncogene Res 1: 149-168, 1987.

Detail Description Paragraph - DETX (219):
[0283] Bolen J B, Veillette A, Schwartz A M, Deseau V, Rosen N: Activation of pp60c-src protein kinase activity in human colon carcinoma. Proc Natl Acad Sci USA 84:2251-2255, 1987.

Detail Description Paragraph - DETX (229):
[0293] Cartwright C A, Kamps M P, Meisler A I, Pipas J M, Eckhart W: p60c-src activation in human colon carcinoma. J Clin Invest 83:2025-2033, 1989.

Detail Description Paragraph - DETX (230):
[0294] Cartwright C A, Meisler A I, Eckhart W: Activation of the pp60c-src protein kinase is an early event in colonic carcinogenesis. Proc Natl Acad Sci USA 87:558-562, 1990.

Detail Description Paragraph - DETX (232):
[0296] Chackalaparampil I, Shalloway D: Altered phosphorylation and activation of pp60c-src during fibroblast mitosis. Cell 52:801-810, 1988.

Detail Description Paragraph - DETX (270):
[0334] Klein N P and Schneider R J. Activation of Src Family Kinases by

Hepatitis B Virus HBx Protein and Coupled Signaling to Ras. Mol Cell Biol 17:6427-6436, 1997.

Detail Description Paragraph - DETX (296):
[0360] Sabe H, Okada M, Nakagawa H, Hanafusa H: Activation of c-src in cells bearing v-Crk and its suppression by CSK. Mol. Cell Biol. 12:4706-4713, 1992.

Detail Description Paragraph - DETX (328):
[0392] Zheng X M, Wang Y, and Pallen C J: Cell transformation and activation of pp60c-src by overexpression of a protein tyrosine phosphatase. Nature 359:336-339, 1992.

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ABSTRACT:

The present invention provides purified human polynucleotides for diagnostics and therapeutics (dithp). Also encompassed are the polypeptides (DITHP) encoded by dithp. The invention also provides for the use of dithp, or complements, oligonucleotides, or fragments thereof in diagnostic assays. The invention further provides for vectors and host cells containing dithp for the expression of DITHP. The invention additionally provides for the use of isolated and purified DITHP to induce antibodies and to screen libraries of compounds and the use of anti-DITHP antibodies in diagnostic assays. Also provided are microarrays containing dithp and methods of use.

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Summary of Invention Paragraph - BSTX (293):

[0291] Many growth factor receptors, including receptors for epidermal growth factor, platelet-derived growth factor, fibroblast growth factor, as well as the growth modulator .alpha.-thrombin, contain intrinsic protein kinase activities. When growth factor binds to the receptor, it triggers the autophosphorylation of a serine, threonine, or tyrosine residue on the receptor. These phosphorylated sites are recognition sites for the binding of other cytoplasmic signaling proteins. These proteins participate in signaling pathways that eventually link the initial receptor activation at the cell surface to the activation of a specific intracellular target molecule. In the case of tyrosine residue autophosphorylation, these signaling proteins contain a common domain referred to as a Src homology (SH) domain. SH2 domains and SH3 domains are found in phospholipase C-.gamma., PI-3-K p85 regulatory subunit, Ras-GTPase activating protein, and pp60.sup.c-src (Lowenstein, E. J. et al. (1992) Cell 70:431-442). The cytokine family of receptors share a different common binding domain and include transmembrane receptors for growth hormone (GM), interleukins, erythropoietin, and prolactin.

Summary of Invention Paragraph - BSTX (769):

[0763] The dithp of the invention may be used for somatic or germline gene therapy. Gene therapy may be performed to (i) correct a genetic deficiency (e.g., in the cases of severe combined immunodeficiency (SCID)-X1 disease characterized by X-linked inheritance (Cavazzana-Calvo, M. et al. (2000) Science 288:669-672), severe combined immunodeficiency syndrome associated with an inherited adenosine deaminase (ADA) deficiency (Blaese, R. M. et al. (1995) Science 270:475-480; Bordignon, C. et al. (1995) Science 270:470-475), cystic fibrosis (Zabner, J. et al. (1993) Cell 75:207-216; Crystal, R. G. et al. (1995) Hun Gene Therapy 6:643-666; Crystal, R. G. et al. (1995) Hum. Gene Therapy 6:667-703), thalassemias, familial hypercholesterolemia, and hemophilia resulting from Factor VIII or Factor IX deficiencies (Crystal, R. G. (1995) Science 270:404-410; Verma, I. M. and Somia, N. (1997) Nature 389:239-242)), (ii) express a conditionally lethal gene product (e.g., in the case of cancers which result from unregulated cell proliferation), or (iii) express a protein which affords protection against intracellular parasites (e.g., against human retroviruses, such as human immunodeficiency virus (IV) (Baltimore, D. (1988) Nature 335:395-396; Poeschla, E. et al. (1996) Proc. Natl. Acad. Sci. USA. 93:11395-11399), hepatitis B or C virus (HBV, HCV); fungal parasites, such as Candida albicans and Paracoccidioides brasiliensis; and protozoan parasites such as Plasmodium falciparum and Trypanosoma cruzi). In the case where a genetic deficiency in dithp expression or regulation causes disease, the

expression of dithp from an appropriate population of transduced cells may alleviate the clinical manifestations caused by the genetic deficiency.

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ABSTRACT:

The present invention provides compositions and methods for utilizing recombinant alphavirus vectors. Also disclosed are compositions and methods for making and utilizing eukaryotic layered vector initiation systems.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of co-pending PCT application US94/10469, filed Sep. 15, 1994. In addition, this application is a continuation-in-part of pending U.S. patent application Ser. No. 08/376,184, filed Jan. 18, 1995, which is a continuation-in-part of pending U.S. patent application Ser. No. 08/348,472, filed Nov. 30, 1994, which is a continuation-in-part of pending U.S. patent application Ser. No. 08/198,450, filed Feb. 18, 1994, which was a continuation-in-part of pending U.S. patent application Ser. No. 08/122,791, filed Sep. 15, 1993.

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Summary of Invention Paragraph - BSTX (24):
[0022] In still other embodiments, the vectors described above include a selected heterologous sequence which may be obtained from a virus selected from the group consisting of influenza virus, HPV, HBV, HCV, EBV, HIV, HSV, FeLV, FIV, Hanta virus, HTLV I, HTLV II and CMV. Within one preferred embodiment, the heterologous sequence obtained from HPV encodes a protein selected from the group consisting of E5, E6, E7 and L1. In yet other embodiments, the vectors described above include a selected heterologous sequence encoding an HIV protein selected from the group consisting of HIV gp120 and gag.

Summary of Invention Paragraph - BSTX (25):
[0023] The selected heterologous sequences described above also may be an antisense sequence, noncoding sense sequence, or ribozyme sequence. In preferred embodiments, the antisense or noncoding sense sequence is selected from the group consisting of sequences which are complementary to influenza virus, HPV, HBV, HCV, EBV, HIV, HSV, FeLV, FIV, Hanta virus, HTLV I, HTLV II, and CMV sequences.

Summary of Invention Paragraph - BSTX (37):
[0035] Within another aspect, HBV-derived and coronavirus-derived packaging cell lines are provided which are suitable for packaging and production of and alphavirus vector. Within one embodiment, an HBV-derived packaging cell line is provided, comprising an expression cassette which directs the expression of HBV core, preS/S, and P proteins. Within another embodiment, a coronavirus-derived packaging cell line is provided, comprising an expression cassette which directs the expression of coronavirus N, M, and S proteins.

Detail Description Paragraph - DETX (61):
[0137] Within related aspects of the present invention, alphavirus cell-specific expression vectors may be constructed to express viral antigens, ribozyme, antisense sequences or immunostimulatory factors such as gamma-interferon (.gamma.-IFN), IL-2 or IL-5 for the targeted treatment of virus infected cell types. In particular, in order to target alphavirus vectors to specific foreign organism or pathogen-infected cells, inverted repeats of the alphavirus vector may be selected to hybridize to any

pathogen-specific RNA, for instance target cells infected by pathogens such as HIV, CMV, HBV, HPV and HSV.

Detail Description Paragraph - DETX (72):

[0148] Another example of an immunomodulatory cofactor is the B7/BB1 costimulatory factor. Briefly, activation of the full functional activity of T cells requires two signals. One signal is provided by interaction of the antigen-specific T cell receptor with peptides which are bound to major histocompatibility complex (MHC) molecules, and the second signal referred to as costimulation, is delivered to the T cell by antigen-presenting cells. Briefly, the second signal is required for interleukin-2 (IL-2) production by T cells and appears to involve interaction of the B7/BB1 molecule on antigen-presenting cells with CD28 and CTLA-4 receptors on T lymphocytes (Linsley et al., J. Exp. Med., 173:721-730, 1991a, and J. Exp. Med., 174:561-570, 1991). Within one embodiment of the invention, B7/BB 1 may be introduced into tumor cells in order to cause costimulation of CD8.sup.+ T cells, such that the CD8.sup.+ T cells produce enough IL-2 to expand and become fully activated. These CD8.sup.+ T cells can kill tumor cells that are not expressing B7 because costimulation is no longer required for further CTL function. Vectors that express both the costimulatory B7/BB1 factor and, for example, an immunogenic HBV core protein, may be made utilizing methods which are described herein. Cells transduced with these vectors will become more effective antigen-presenting cells. The HBV core-specific CTL response will be augmented from the fully activated CD8.sup.+ T cell via the costimulatory ligand B7/BB1.

Detail Description Paragraph - DETX (109):

[0185] Within other aspects of the present invention, alphavirus vector constructs are provided which direct the expression of immunogenic portions of antigens from foreign organisms or other pathogens. Representative examples of such antigens include bacterial antigens (e.g., E. coli, streptococcal, staphylococcal, mycobacterial, etc.), fungal antigens, parasitic antigens, and viral antigens (e.g., influenza virus, Human Immunodeficiency Virus ("HIV"), Hepatitis A, B and C Virus ("HAV", "HBV" and "HCV", respectively), Human Papiloma Virus ("HPV"), Epstein-Barr Virus ("EBV"), Herpes Simplex Virus ("HSV"), Hantavirus, TTLV I, HTLV II and Cytomegalovirus ("CMV"). As utilized within the context of the present invention, "immunogenic portion" refers to a portion of the respective antigen which is capable, under the appropriate conditions, of causing an immune response (i.e., cell-mediated or humoral). "Portions" may be of variable size, but are preferably at least 9 amino acids long, and may include the entire antigen. Cell-mediated immune responses may be mediated through Major Histocompatability Complex ("MHC") class I presentation, MHC Class II presentation, or both.

Detail Description Paragraph - DETX (110):

[0186] Within one aspect of the invention, alphavirus vector constructs are provided which direct the expression of immunogenic portions of Hepatitis B antigens. Briefly, the Hepatitis B genome is comprised of circular DNA of about 3.2 kilobases in length and has been well characterized (Tiollais et al., Science 213:406-411, 1981; Tiollais et al., Nature 317:489-495, 1985; and Ganem and Varmus, Ann. Rev. Biochem. 56:651-693, 1987; see also EP 0 278,940, EP 0 241,021, WO 88/10301, and U.S. Pat. Nos. 4,696,898 and 5,024,938, which are hereby incorporated by reference). The Hepatitis B virus presents several different antigens, including among others, three HB "S" antigens (HBsAgs), an HBc antigen (HBcAg), an HBe antigen (HBeAg), and an HBx antigen (HBxAg) (see Blum et al., TIG 5(5):154-158, 1989). Briefly, the HBeAg results from proteolytic cleavage of a P22 pre-core intermediate and is secreted from the cell. HBeAg is found in serum as a 17 kD protein. The HBcAg is a protein of 183 amino acids, and the HBxAg is a protein of 145 to 154 amino acids,

depending on subtype.

Detail Description Paragraph - DETX (112):

[0188] As will be evident to one of ordinary skill in the art, various immunogenic portions of the above-described S antigens may be combined in order to induce an immune response when administered by one of the alphavirus vector constructs described herein. In addition, due to the large immunological variability that is found in different geographic regions for the S open reading frame of HBV, particular combinations of antigens may be preferred for administration in particular geographic regions. Briefly, epitopes that are found in all human hepatitis B virus S samples are defined as determinant "a". Mutually exclusive subtype determinants, however, have also been identified by two-dimensional double immunodiffusion (Ouchterlony, Progr. Allergy 5:1, 1958). These determinants have been designated "d" or "y" and "w" or "r" (LeBouvier, J. Infect. 123:671, 1971; Bancroft et al., J. Immunol. 109:842, 1972; and Courouce et al., Bibl. Haematol. 42:1-158, 1976). The immunological variability is due to single nucleotide substitutions in two areas of the hepatitis B virus S open reading frame, resulting in the following amino acid changes: (1) exchange of lysine-122 to arginine in the Hepatitis B virus S open reading frame causes a subtype shift from d to y, and (2) exchange of arginine-160 to lysine causes the shift from subtype r to w. In Africans, subtype ayw is predominant, whereas in the U.S. and northern Europe the subtype adw.sub.2 is more abundant (Molecular Biology of the Hepatitis B Virus, McLachlan (ed.), CRC Press, 1991). As will be evident to one of ordinary skill in the art, it is generally preferred to construct a vector for administration which is appropriate to the particular hepatitis B virus subtype which is prevalent in the geographical region of administration. Subtypes of a particular region may be determined by two-dimensional double immunodiffusion or, preferably, by sequencing the S open reading frame of HBV virus isolated from individuals within that region.

Detail Description Paragraph - DETX (113):

[0189] Also presented by HBV are pol ("HBV pol"), ORF 5, and ORF 6 antigens. Briefly, the polymerase open reading frame of HBV encodes reverse transcriptase activity found in virions and core-like particles in infected livers. The polymerase protein consists of at least two domains: the amino terminal domain which encodes the protein that primes reverse transcription, and the carboxyl terminal domain which encodes reverse transcriptase and RNase H activity. Immunogenic portions of HBV pol may be determined utilizing methods described herein (e.g., below and in Example 13), utilizing alphavirus vector constructs described below, and administered in order to generate an immune response within a warm-blooded animal. Similarly, other HBV antigens, such as ORF 5 and ORF 6 (Miller et al., Hepatology 9:322-327, 1989) may be expressed utilizing alphavirus vector constructs as described herein. Representative examples of alphavirus vector constructs utilizing ORF 5 and ORF 6 are set forth below in the examples.

Detail Description Paragraph - DETX (129):

[0205] A wide variety of vector systems may be utilized as the first layer of the eukaryotic layered vector initiation system, including for example, viral vector constructs developed from DNA viruses such as those classified in the Poxviridae, including for example canary pox virus or vaccinia virus (e.g., Fisher-Hoch et al., PNAS 86:317-321, 1989; Flexner et al., Ann. N.Y. Acad. Sci. 569:86-103, 1989; Flexner et al., Vaccine 8:17-21, 1990; U.S. Pat. Nos. 4,603,112, 4,769,330 and 5,017,487; WO 89/01973); Papoviridae such as BKV, JCV or SV40 (e.g., Mulligan et al., Nature 277:108-114, 1979); Adenoviridae, such as adenovirus (e.g., Berkner, Biotechniques 6:616-627, 1988; Rosenfeld et al., Science 252:431-434, 1991); Parvoviridae, such as adeno-associated virus (e.g., Samulski et al., J. Vir. 63:3822-3828, 1989; Mendelson et al., Virol.

166:154-165, 1988; PA 7/222,684); Herpesviridae, such as Herpes Simplex Virus (e.g., Kit, Adv. Exp. Med. Biol. 215:219-236, 1989); and Hepadnaviridae (e.g., HBV), as well as certain RNA viruses which replicate through a DNA intermediate, such as the Retroviridae (see, e.g., U.S. Pat. No. 4,777,127, GB 2,200,651, EP 0,345,242 and WO91/02805; Retroviridae include leukemia in viruses such as MoMLV and immunodeficiency viruses such as HIV, e.g., Poznansky, J. Virol. 65:532-536, 1991).

Detail Description Paragraph - DETX (149):

[0225] Within other aspects of the present invention, compositions and methods are provided for administering an alphavirus vector construct which is capable of preventing, inhibiting, stabilizing or reversing infectious, cancerous, auto-immune or immune diseases. Representative examples of such diseases include viral infections such as HIV, HBV HTLV I, HTLV II, CMV, EBV and HPV, melanomas, diabetes, graft vs. host disease, Alzheimer's disease and heart disease.

Detail Description Paragraph - DETX (500):

[0569] Using techniques described above, the lacZ gene encoding the .beta.-galactosidase reporter protein was cut from the plasmid pSV-.beta.-galactosidase (PROMEGA CORP, Madison, Wis.) and substituted into the ELVIS-luc plasmid DNA vector in place of luciferase. To examine in vivo gene expression from ELVIS vectors, Balb/c mice and rats are injected intramuscularly (i.m.) with ELVIS-.beta.-gal or ELVIS-luc plasmid DNA vectors. FIG. 24 demonstrates the in vivo expression of .beta.-galactocidase in muscle tissue taken from a rat and stained with X-gal at three days post i.m. injection. Mice injected with ELVIS-.beta.-gal also demonstrate positively staining blue muscle fibers. Luciferase expression levels from muscle which were between 75- and 300-fold higher than control levels were detected in 3/4 Balb/c mice at two days post i.m. inoculation with ELVIS-luc plasmid. In other experiments, C3H/HeN mice were injected i.m. with ELVIS vectors expressing either the hepatitis B virus core (HBV-core) or hepatitis B virus e (HBV-e) proteins. Using ELISA detection systems, both HBV-core- and HBV-e-specific IgG antibodies were detected in serum samples collected from the mice 10 days following the second injection with the vectors. These experiments demonstrate that Sindbis-derived DNA vectors are able to express foreign genes in vivo, in rat and mouse muscle.

Detail Description Paragraph - DETX (621):

[0684] In the derivation of alphavirus vector packaging and producer cell lines, many approaches are outlined to control the expression of viral genes, such that producer cell lines stably transformed with both vector and vector packaging cassettes, can be derived. These approaches include inducible and/or cellular differentiation sensitive promoters, antisense structural genes, heterologous control systems, and mosquito or other cells in which viral persistent infections are established. Regardless of the final configuration for the alphavirus vector producer cell line, the ability to establish persistent infection, or at least delay cell death as a result of viral gene expression, may be enhanced by inhibiting apoptosis. For example, the DNA tumor viruses, including adenovirus, HPV, SV40, and mouse polyomavirus (Py), transform cells in part, by binding to, and inactivating, the retinoblastoma (Rb) gene product p105 and its closely related gene product, p107, and other gene products involved in the control of the cell cycle including cyclin A, p33.sup.cdk2 and p34.sup.cdc2. All of these viruses, except for Py, encode gene products which bind to and inactivate p53. Uniquely, Py encodes middle T antigen (mT) which binds to and activates the membrane tyrosine kinase, src, and also phosphatidylinositol-3-kinase, which is required for the full transformation potential of this virus (Talmage et al., Cell 59:55-65, 1989). The binding to and inactivation of the Rb and p53 recessive oncogene products

prevents cells transformed by these DNA tumor viruses from entering the apoptotic pathway. It is known that p53 is able to halt the division of cells, in part by inhibiting the expression of proteins associated with cellular proliferation, including c-fos, hsc70, and bcl-2 (Miyashita et al., Cancer Research 54:3131-3135,1994).

Detail Description Paragraph - DETX (758):
[0817] In another embodiment, a RNA packaging signal derived from another virus is inserted into the alphavirus vector to allow packaging by the structural proteins of that corresponding virus. For example, the 137 nt. packaging signal from hepatitis B virus, located between nts. 3134 and 88 and spanning the precore/core junction (Junker-Niepmann et al. EMBO J. 9:3389, 1990), is amplified from an HBV template using two oligonucleotide primers. PCR is performed using a standard three temperature cycling protocol, plasmid pHBV1.1 (Junker-Niepmann et al. EMBO J. 9:3389, 1990) as the template, and the following oligonucleotide pair, each of which contain 20 nucleotides complementary to the HBV sequence and flanking ApaI recognition sequences:

Detail Description Paragraph - DETX (761):
[0820] Following amplification, the PCR amplicon is digested with ApaI and purified from a 1.5% agarose gel using MERMAID.TM. (Bio101). Sindbis vector plasmid pKSSINdIJRsjrc (Example 3) also is digested with ApaI, under limited conditions to cleave at only one of its two sites, followed by treatment with CIAP, purification from a 1% agarose gel, and ligation with the above-synthesized HBV amplicon, to produce a construct designated pKSSINhbvJR. Other alphavirus vectors (see Example 3) are readily modified in a similar manner. Cell lines which express the HBV core, preS/S, and P proteins necessary for packaging of the RNA sequence are derived by modification of helper plasmid pCH3143 (Junker-Niepmann et al., EMBO J. 9:3389, 1990) to include a selectable marker. An expression cassette containing the neomycin resistance marker is obtained by digestion of plasmid pBK-RSV (Stratagene)-with Mst II and blunt-ending with Klenow fragment. The selectable marker is then ligated into any one of several unique sites within pCH3143 that have been digested and their termini made blunt. The resulting construct is transfected into a desired cell line, for example, mouse hepatoma line Hepa1-6 (ATCC #CRL1830), and selected using the drug G418, as described in Example 7. Introduction of the pKSSINhbvJR vector, or related RNA- or DNA-based alphavirus vectors, results in the production of packaged vector particles with the same hepatotropism as HBV.

Detail Description Paragraph - DETX (765):
[0824] Following amplification, the PCR amplicon is digested with ApaI, purified from a 1.5% agarose gel using MERMAID.TM., and ligated into pKSSINdIJRsjrc, prepared as described for HBV. The resulting construct is designated pKSSINmhvJR. Other alphavirus vectors (see Example 3) are readily modified in a similar manner. Packaging of vectors modified with this MHV sequence is accomplished by using expression cassettes which produce each of the required coronavirus structural proteins: nucleocapsid (N protein; Armstrong et al., NAR 11:883, 1983); membrane (M protein, Armstrong et al., Nature 308:751, 1984); and spike (S protein, Luytjes et al., Virology 161:479, 1987). Preferably, these proteins are inserted into the vector-inducible pVGELVSdI-linker plasmid (described previously in this example) and selected for with the G418 drug following transfection into the appropriate cell type. Other expression methodologies (see Example 7) may also be readily utilized. Addtional coronaviruses, for example, human coronaviruses OC43 (ATCC #VR-759) and. 229E (ATCC #VR-740), can be readily used in place of MHV to produce packaged recombinant alphavirus particles which show tropism for cells in the respiratory tract.

Detail Description Paragraph - DETX (770):
[0829] In each case, the packaging sequences from HBV, coronavirus, retrovirus, or any other virus, also may be incorporated into alphavirus vectors at locations other than those outlined above, provided the location is not present in the subgenomic transcript. For example, the next most preferable site of insertion is the carboxy-terminal region of nonstructural protein 3, which has been shown to be highly variable in both length and sequence among all alphaviruses for which sequence information is available. Further, these applications are not limited by the ability to derive the corresponding packaging cell lines, as the necessary structural proteins also may be expressed using any of the altenative approaches described in Example 8.

Detail Description Paragraph - DETX (805):
Generation of Vector Constructs which Express HBV Antigens for the Induction of an Immune Response

Detail Description Paragraph - DETX (806):
[0856] A. Isolation of HBV E/Core Sequence

Detail Description Paragraph - DETX (809):
[0859] 1. Site-Directed Mutagenesis of HBV E/Core Sequence Utilizing PCR

Detail Description Paragraph - DETX (816):
[0866] The second primer corresponds to the anti-sense nucleotide sequence from SK+ plasmid polylinker and contains a Cla I site 135 bp downstream of the stop codon of the HBV precore/core coding region.

Detail Description Paragraph - DETX (819):
[0869] The second primer sequence corresponds to the anti-sense nucleotide sequence from the SK+ plasmid polylinker and contains a Cla I site 135 bp downstream of the stop codon of the HBV precore/core coding region.

Detail Description Paragraph - DETX (820):
[0870] The first PCR reaction corrects the deletion in the antisense strand and the second reaction corrects the deletion in the sense strands. PCR reactions one and two correct the mutation from CC to CCA which occurs in codon 79 and a base pair substitution from TCA to TCT in codon 81. Primer 1 contains two consecutive Xho I sites 10 bp upstream of the ATG codon of HBV e coding region and primer 4 contains a Cla I site 135 bp downstream of the stop codon of HBV precore/core coding region. The products of the first and second PCR reactions are extended in a third PCR reaction to generate one complete HBV precore/core coding region with the correct sequence.

Detail Description Paragraph - DETX (823):
[0873] 2. Isolation of HBV Core Sequence

Detail Description Paragraph - DETX (831):
[0881] The PCR product from the third reaction yields the correct sequence for HBV precore/core coding region.

Detail Description Paragraph - DETX (832):
[0882] To isolate HBV core coding region, a primer is designed to introduce the Xho I restriction site upstream of the ATG start codon of the core coding region, and eliminate the 29 amino acid leader sequence of the HBV precore coding region. In a fourth reaction, the HBV core coding region is produced using the PCR product from the third reaction and the following two primers.

Detail Description Paragraph - DETX (834):
[0884] The second primer corresponds to the anti-sense nucleotide sequence

for the T-3 promoter present in the SK+ HBe plasmid. The approximately 600 bp PCR product from the fourth PCR reaction contains the HBV core coding region and novel Xho I restriction sites at the 5' end and Cla I restriction sites at the 3' end that was present in the multicloning site of SK+ HBe plasmid.

Detail Description Paragraph - DETX (835):
[0885] Following the fourth PCR reaction, the solution is transferred into a fresh 1.5 ml microfuge tube. Fifty microliters of 3 M sodium acetate is added to this solution followed by 500 .mu.l of chloroform:isoamyl alcohol (24:1). The mixture is vortexed and then centrifuged at 14,000 rpm for 5 minutes. The aqueous phase is transferred to a fresh microfuge tube and 1.0 ml 100% EtOH is added. This solution is incubated at -20.degree. C. for 4.5 hours, and then centrifuged at 10,000 rpm for 20 minutes. The supernatant is decanted, and the pellet rinsed with 500 .mu.l of 70% EtOH. The pellet is dried by centrifugation at 10,000 rpm under vacuum and then resuspended in 10 .mu.l deionized H.sub.2O. One microliter of the PCR product is analyzed by 1.5% agarose gel electrophoresis. The approximately 600 bp Xho I-Cla I HBV core PCR fragment is cloned into the Xho I-Cla I site of SK+ plasmid. This plasmid is designated SK+HBc.

Detail Description Paragraph - DETX (836):
[0886] 3. Isolation of HBV X Antigen

Detail Description Paragraph - DETX (839):
[0889] 4. Construction of Sindbis Vectors Expressing HBVE, HBV Core and HBV X

Detail Description Paragraph - DETX (841):
[0891] Construction of a Sindbis vector expressing the HBV core sequence is accomplished by digestion of plasmid SK+HBc (described above) with Xho I and Xba I. The HBc fragment is isolated by agarose gel electrophoresis, purified by GENECLEAN.TM. and ligated into pKSSINBV at the Xho I and Xba I sites. This Sindbis-HBc vector is designated pKSSIN-HBc.

Detail Description Paragraph - DETX (842):
[0892] Construction of a Sindbis vector expressing the HBV-X antigen sequence is accomplished by digesting the plasmid SK-X Ag with Xho I and Xba I to release a cDNA fragment encoding HBV-X sequences. The fragment is isolated by agarose gel electrophoresis, purified using GENECLEAN.TM., and inserted into pKSSINBV, pre-treated with Xho I and Xba I enzymes. This Sindbis-HBx vector is designated pKSIN-HBx.

Detail Description Paragraph - DETX (843):
[0893] The above Sindbis HBV expressing vectors may also be modified to coexpress a selectable drug resistance marker dependent on the requirements of the experiment or treatment of the vector infected cells. In particular, any of the above Sindbis HBV expression vectors described may also be designed to coexpress G418 resistance. This is accomplished by incorporating an internal ribosomal entry site (Example 5) followed by the bacterial neomycin phosphotransferase gene placed 3' of the HBV coding sequences and 5' of the terminal 3' end of the vector using the multiple cloning site of the vector. These G418 resistant vector constructs can be used for selecting vector infected cells for the generation of HBV specific CTL targets in the following sections.

Detail Description Paragraph - DETX (846):
[0896] Cell lysates from cells infected by any of the HBV expressing vectors are made by washing 1.0.times.10.sup.7 cultured cells with PBS, resuspending the cells to a total volume of 600 .mu.l in PBS, and sonicating for two

5-second periods at a setting of 30 in a Branson sonicator, Model 350 (Fisher, Pittsburgh, Pa.) or by freeze thawing three times. Lysates are clarified by centrifugation at 10,000 rpm for 5 minutes.

Detail Description Paragraph - DETX (848):
[0898] As shown in FIG. 16, using these procedures approximately 100-200 ng/ml HBV e antigen is expressed in the cell lysates and 300-400 ng/ml HBV e antigen is secreted from BHK cells infected with the Sin BV HB e vector.

Detail Description Paragraph - DETX (849):
[0899] As shown in FIG. 17, using these procedures, approximately 40 ng/ml HBV core antigen is expressed in the cell lysates from 10.sup.6 BHK cells infected with the Sin BV HBcore. Mouse fibroblast cells infected with the recombinant HBcore Sindbis vector express 6-7 fold higher HBV core protein levels than the recombinant HBcore retroviral vector transduced cells (WO 93/15207). As shown in FIG. 18, using these procedures, approximately 12-14 ng/ml HBV core antigen is expressed in the cell lysates from 10.sup.6 L-M(TK-) cells infected with the SinBVHBcore vector as compared to the approximately 2 ng/ml HBV core antigen expressed from recombinant HBcore retroviral vector transducer cells.

Detail Description Paragraph - DETX (857):
[0907] Using these procedures, it can be shown that CTLs to HBV e antigen can be induced.

Detail Description Paragraph - DETX (861):
[0911] Lymphoblastoid cell lines (LCL) are established for each patient by infecting (transforming) their B-cells with fresh Epstein-Barr virus (EBV) taken from the supernatant of a 3-week-old culture of B95-8, EBV transformed marmoset leukocytes (ATCC CRL 1612). Three weeks after EBV-transformation, the LCL are infected with Sindbis vector expressing HBV core or e antigen and G418 resistance. Vector infection of LCL is accomplished by infecting LCL cells with packaged alphavirus vector particles produced from the appropriaste cell line The culture medium consists of RPMI 1640, 20% heat inactivated fetal bovine serum (Hyclone, Logan, Utah), 5.0 mM sodium pyruvate and 5.0 mM non-essential amino acids. Infected LCL cells are selected by adding 800 .mu.g/ml G418. The Jurkat A2/K.sup.b cells (L. Sherman, Scripps Institute, San Diego, Calif.) are infected essentially as described for the infection of LCL cells.

Detail Description Paragraph - DETX (865):
[0915] Humoral immune responses in mice specific for HBV core and e antigens are detected by ELISA. The ELISA protocol utilizes 100 .mu.g/well of recombinant HBV core and recombinant HBV e antigen (Biogen, Geneva, Switzerland) to coat 96-well plates. Sera from mice immunized with vector expressing HBV core or HBV e antigen are then serially diluted in the antigen-coated wells and incubated for 1 to 2 hours at room temperature. After incubation, a mixture of rabbit anti-mouse IgG1, IgG2a, IgG2b, and IgG3 with equivalent titers is added to the wells. Horseradish peroxidase ("HRP")-conjugated goat anti-rabbit anti-serum is added to each well and the samples are incubated for 1 to 2 hours at room temperature. After incubation, reactivity is visualized by adding the appropriate substrate. Color will develop in wells that contain IgG antibodies specific for HBV core or HBV e antigen.

Detail Description Paragraph - DETX (867):
[0917] Antigen induced T-helper activity resulting from two or three injections of Sindbis vector expressing HBV core or e antigen, is measured in vitro. Specifically, splenocytes from immunized mice are restimulated in vitro

at a predetermined ratio with cells expressing HBV core or e antigen or with cells not expressing HBV core or e antigen as a negative control. After five days at 37.degree. C. and 5% CO.sub.2 in RPMI 1640 culture medium containing 5% FBS, 1.0 mM sodium pyruvate and 10.sup.-5 2-mercaptoethanol, the supernatant is tested for IL-2 activity. IL-2 is secreted specifically by T-helper cells stimulated by HBV core or e antigen, and its activity is measured using the CTL clone, CTLL-2 (ATCC TIB 214). Briefly, the CTLL-2 clone is dependent on IL-2 for growth and will not proliferate in the absence of IL-2. CTLL-2 cells are added to serial dilutions of supernatant test samples in a 96-well plate and incubated at 37.degree. C. and 5%, CO.sub.2 for 3 days. Subsequently, 0.5.mu. Ci .sup.3H-thymidine is added to the CTLL-2 cells. 0.5 Ci .sup.3H-thymidine is incorporated only if the CTLL-2 cells proliferate. After an overnight incubation, cells are harvested using a PHD cell harvester (Cambridge Technology Inc., Watertown, Mass.) and counted in a Beckman beta counter. The amount of IL-2 in a sample is determined from a standard curve generated from a recombinant IL-2 standard obtained from Boehringer Mannheim (Indianapolis, Ind.).

Detail Description Paragraph - DETX (871):

[0921] The mouse system may also be used to evaluate the induction of humoral and cell-mediated immune responses with direct administration of Sindbis vector encoding HBV core or e antigen. Briefly, six- to eight-week-old female C3H/He mice are injected intramuscularly (i.m.) with 0.1 ml of reconstituted (with sterile deionized, distilled water) or intraperitoneally (ip) with 1.0 ml of lyophilized HBV core or HBV e expressing Sindbis vector. Two injections are given one week apart. Seven days after the second injection, the animals are sacrificed. Chromium release CTL assays are then performed essentially as described in Example 13E 1.a.

Detail Description Paragraph - DETX (873):

[0923] The data generated in the mouse system described above is used to determine the protocol of administration of vector in chimpanzees chronically infected with hepatitis B virus. Based on the induction of HBV-specific CTLs in mice, the subjects in chimpanzee trials will receive four doses of vector encoding core or e antigen at 7 day intervals given in two successively escalating dosage groups. Control subjects will receive a placebo comprised of formulation media. The dosage will be either 10.sup.7 or 10.sup.8 pfu given in four 1.0 ml injections i.m. on each injection day. Blood samples will be drawn on days 0, 14, 28, 42, 56, 70, and 84 in order to measure serum alanine aminotransferase (ALT) levels, the presence of hepatitis B e antigen, the presence of antibodies directed against the hepatitis B e antigen, serum HBV DNA levels and to assess safety and tolerability of the treatment. The hepatitis B e antigen and antibodies to HB e antigen is detected by Abbott HB e rDNA EIA kit (Abbott Laboratories Diagnostic Division, Chicago, Ill.) and the serum HBV DNA levels is determined by the Chiron bDNA assay. Efficacy of the induction of CTLs against hepatitis B core or e antigen can be determined as in Example 13E 1.c.

Detail Description Paragraph - DETX (874):

[0924] Based on the safety and efficacy results from the chimpanzee studies, the dosage and inoculation schedule is determined for administration of the vector to subjects in human trials. These subjects are monitored for serum ALT levels, presence of HBV e antigen, the presence of antibodies directed against the HBV e antigen and serum HBV DNA levels essentially as described above. Induction of human CTLs against hepatitis B core or e antigen is determined as in Example 13E 1.c.

Detail Description Paragraph - DETX (875):

[0925] G. Generation of ELVIS Vector Constructs which Express HBV Antigens

for the Induction of an Immune Response

Detail Description Paragraph - DETX (876):
[0926] 1. Construction of ELVIS Vectors Expressing HBVe-c, HBV Core and HBV X

Detail Description Paragraph - DETX (877):
[0927] Construction of an ELVIS vector expressing the HBV e antigen is accomplished by digesting the SK.sup.+HB e-c plasmid with Xho I and Not I to release the cDNA fragment encoding HBVe-c sequences. The fragment is then isolated by agarose gel electrophoresis, purified using GENECLEAN.TM., and inserted into pVGELVIS-SINBV-linker vector, previously prepared by digestion with Xho I and Not I. This construct is designated pVGELVIS-HBe.

Detail Description Paragraph - DETX (878):
[0928] The HBcore PCR product described previously is digested with Xho I and Cla I, isolated by agarose gel electrophoresis, purified using GENECLEAN.TM., and ligated into SK+II (Bluescript, Stratagene, Calif.) digested with Xho I and Cla I. This construct is designated SK+HBcore. Construction of the ELVIS vector expressing the HBV core sequence is accomplished by digesting the SK.sup.+HBcore plasmid with Xho I and Not I to release the cDNA fragment encoding HBVcore sequences. The fragment is then isolated by agarose gel electrophoresis, purified using GENECLEAN.TM., and inserted into pVGELVIS-SINBV-linker vector, prepared by digestion with Xho I and Not I. This construct is designated pVGELVIS-HBcore.

Detail Description Paragraph - DETX (879):
[0929] Construction of the ELVIS vector expressing the HBV-X antigen sequence is accomplished by digesting the plasmid SK-X Ag with Xho I and Not I to release the cDNA fragment encoding HBV-X sequences. The fragment is then isolated by agarose gel electrophoresis, purified using GENECLEAN, and inserted into the pVGELVIS-SINBV-linker vector, prepared by digestion with Xho I and Not I. This construct is designated pVGELVIS-HBX.

Detail Description Paragraph - DETX (880):
[0930] Any of the above three constructs can be used for selecting vector infected cells for the generation of HBV specific CTL targets in the following sections.

Detail Description Paragraph - DETX (882):
[0932] The pVGELVIS-HBe plasmid DNA is isolated and purified, and 2 ug of pVGELVIS-HBe DNA is complexed with 10 ul of LIPOFECTAMINE.TM. and transfected into 2.times.10.sup.5 BHK cells contained in 35 mM petri plates. Two days post-transfection, supernatants and whole cell lysates were collected and an ELISA assay (see below) was used to determine the amount of expressed HBV-e antigen.

Detail Description Paragraph - DETX (885):
[0935] As shown in FIG. 19, using these procedures, approximately 2 ng/ml HBV e antigen is expressed in the cell lysates and also secreted from BHK cells transfected with different clones of the pVGELVISHBe plasmid.

Detail Description Paragraph - DETX (889):
[0939] The mouse model system is also used to evaluate the induction of humoral and cell-mediated immune responses following direct administration of ELVIS vector expressing HBV core or e antigen. Briefly, six- to eight-week-old female Balb/c, C57B1/6, C3H/He mice (Charles River, Mass.) and HLA A2.1 transgenic mice (V. Engelhard, Charlottesville, Va.) are injected intramuscularly (i.m.) with, for example, 50 ug or greater, pVGELVIS-HBcore,

pVGELVIS-HBVe or pVGELVIS-HBX vector DNA. Two injections are given one week apart. Seven or fourteen days after the second injection, the animals are sacrificed. Chromium release CTL assays are then performed essentially as described in Example 13E 1.a. Detection of humoral immune responses in mice is performed essentially as described in Example 13E 2 and detection of T cell proliferation in mice is performed essentially as described in Example 13E 3.

Claims Text - CLTX (11):

10. The eukaryotic layered vector initiation system according to either of claims 1 or 2, wherein said selected heterologous sequence is obtained from a virus selected from the group consisting of influenza virus, respiratory syncytial virus, HPV, HBV, HCV, EBV, HIV, HSV, FeLV, FIV, Hantavirus, HTLV I, HTLV II, and CMV.

Claims Text - CLTX (13):

12. The eukaryotic layered vector initiation system according to either of claims 1 or 2, wherein said antisense or non-coding sense sequence is selected from the group consisting of sequences which are complementary to influenza virus, respiratory syncytial virus, HPV, HBV, HCV, EBV, HIV, HSV, and CMV sequences.

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TITLE: Inhibitors of c-Jun N-terminal kinases (JNK) and other protein kinases

PUBLICATION-DATE: February 5, 2004

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ABSTRACT:

The present invention provides compounds of formula I: 1
or a pharmaceutically acceptable derivative o thereof, wherein A, B, R.sup.a, R.sup.1, R.sup.2, R.sup.3, and R.sup.4 are as described in the specification. These compounds are inhibitors of protein kinase, particularly inhibitors of JNK, a mammalian protein kinase involved cell proliferation, cell death and response to extracellular stimuli; Lck and Src kinase. The invention also relates to methods for producing these inhibitors. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of priority to co-pending U.S. provisional applications No. 60/283,621 filed Apr. 13, 2001, No. 60/329,440 filed Oct. 14, 2001 and No. 60/292,974 filed May 23, 2001.

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Summary of Invention Paragraph - BSTX (19):
[0018] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J., 18, 5019, (1999) and Klein et al., Mol. Cell. Biol., 17, 6427 (1997).

Summary of Invention Paragraph - BSTX (22):
[0021] Accordingly, there is still a great need to develop potent inhibitors of JNKs and Src family kinases that are useful in treating various conditions associated with JNK and Src activation.

Summary of Invention Paragraph - BSTX (212):
[0209] The activity of the compounds of this invention as kinase inhibitors may be assayed in vitro, in vivo or in a cell line. In vitro assays include assays that determine inhibition of either the kinase activity or ATPase activity of activated enzyme, for example JNK, Lck, or Src. Alternate in vitro assays quantitate the ability of the inhibitor to bind to JNK, Lck, or Src and may be measured either by radiolabelling the inhibitor prior to binding, isolating the inhibitor/JNK, inhibitor/Lck, or inhibitor/Src complex and determining the amount of radiolabel bound, or by running a competition experiment where new compounds are incubated with JNK, Lck, or Src bound to known radioligands. One may use any type or isoform of JNK, Lck, or Src, depending upon which JNK, Lck, or Src type or isoform is to be inhibited. The details of the conditions used for the enzymatic assays are set forth in the Examples hereinbelow.

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TITLE: Receptors

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ABSTRACT:

The invention provides human receptors (REPTR) and polynucleotides which identify and encode REPTR. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides

methods for diagnosing, treating, or preventing disorders associated with aberrant expression of REPTR.

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Summary of Invention Paragraph - BSTX (10):
[0008] Receptors bound to growth factors trigger intracellular signal transduction pathways which activate various downstream effectors that regulate gene expression, cell division, cell differentiation, cell motility, and other cellular processes. Many growth factor receptors, including receptors for epidermal growth factor, platelet-derived growth factor, fibroblast growth factor, and the growth modulator .alpha.-thrombin, contain intrinsic protein linase activities. These signaling proteins contain a common domain referred to as a Src homology (SH) domain. SH2 domains and SH3 domains are found in phospholipase C-.gamma., PI-3-K p85 regulatory subunit, Ras-GTPase activating protein, and pp60.sup.c-src (Lowenstein, E. J. et al. (1992) Cell 70:431-442). The cytokine family of receptors share a different common binding domain and include transmembrane receptors for growth hormone (GH), interleukins, erythropoietin, and prolactin. Other receptors and second messenger-binding proteins have intrinsic serine/threonine protein kinase activity. These include activin/TGF-.beta./BMP-superfamily receptors, calcium- and diacylglycerol-activated/phospholipid-dependant protein kinase (PK-C), and RNA-dependant protein kinase (PK-R). In addition, other serine/threonine protein kinases, including nematode Twitchin, have fibronectin-like, immunoglobulin C2-like domains.

Summary of Invention Paragraph - BSTX (208):
[0200] In another embodiment of the invention, polynucleotides encoding REPTR may be used for somatic or germline gene therapy. Gene therapy may be performed to (i) correct a genetic deficiency (e.g., in the cases of severe combined immunodeficiency (SCID)-X1 disease characterized by X-linked inheritance (Cavazzana-Calvo, M. et al. (2000) Science 288:669-672), severe combined immunodeficiency syndrome associated with an inherited adenosine deaminase (ADA) deficiency (Blaese, R. M. et al. (1995) Science 270:475-480; Bordignon, C. et al. (1995) Science 270:470-475), cystic fibrosis (Zabner, J. et al. (1993) Cell 75:207-216; Crystal, R. G. et al. (1995) Hum. Gene Therapy 6:643-666; Crystal, R. G. et al. (1995) Hum. Gene Therapy 6:667-703), thalassamias, familial hypercholesterolemia, and hemophilia resulting from Factor VM or Factor IX deficiencies (Crystal, R. G. (1995) Science 270:404-410; Verma, I. M. and N. Somia (1997) Nature 389:239-242)), (ii) express a conditionally lethal gene product (e.g., in the case of cancers which result from unregulated cell proliferation), or (iii) express a protein which affords protection against intracellular parasites (e.g., against human retroviruses, such as human immunodeficiency virus (HIV) (Baltimore, D. (1988) Nature 335:395-396; Poeschla, E. et al. (1996) Proc. Natl. Acad. Sci. USA. 93:11395-11399), hepatitis B or C virus (HBV, HCV); fungal parasites, such as Candida albicans and Paracoccidioides brasiliensis; and protozoan parasites such as Plasmodium falciparum and Trypanosoma cruzi). In the case where a genetic deficiency in REPTR expression or regulation causes disease, the expression of REPTR from an appropriate population of transduced cells may alleviate the clinical manifestations caused by the genetic deficiency.

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non-provisional-of-provisional 60147500 19990805 US

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US-CL-CURRENT: 435/6, 435/183 , 435/320.1 , 435/325 , 435/69.1 , 435/7.1 , 530/388.26 , 536/23.2 , 800/8

ABSTRACT:

The present invention provides purified human polynucleotides for diagnostics and therapeutics (dithp). Also encompassed are the polypeptides (DITHP) encoded by dithp. The invention also provides for the use of dithp, or complements, oligonucleotides, or fragments thereof in diagnostic assays. The invention further provides for vectors and host cells containing dithp for the expression of DITHP. The invention additionally provides for the use of isolated and purified DITHP to induce antibodies and to screen libraries of compounds and the use of anti-DITHP antibodies in diagnostic assays. Also provided are microarrays containing dithp and methods of use.

[0001] This application is a continuation-in-part of U.S. Ser. No. 09/980,285, filed Nov. 30, 2001, which is the National Stage of International Application No. PCT/US00/15404, filed May 31, 2000, and published in English as WO 00/73509 on Dec. 7, 2000, which claims the benefit under 35 U.S.C. .sctn. 119(e) of provisional applications U.S. Ser. No. 60/147,500, filed Aug. 5, 1999, U.S. Ser. No. 60/147,542, filed Aug. 5, 1999, U.S. Ser. No. 60/147,541, filed Aug. 5, 1999, U.S. Ser. No. 60/147,824, filed Aug. 5,

1999, U.S. Ser. No. 60/147,547, filed Aug. 5, 1999, U.S. Ser. No. 60/147,530, filed Aug. 5, 1999, U.S. Ser. No. 60/147,536, filed Aug. 5, 1999, U.S. Ser. No. 60/147,520, filed Aug. 5, 1999, U.S. Ser. No. 60/147,527, filed Aug. 5, 1999, U.S. Ser. No. 60/147,549, filed Aug. 5, 1999, U.S. Ser. No. 60/147,377, filed Aug. 4, 1999, U.S. Ser. No. 60/147,436, filed Aug. 4, 1999, U.S. Ser. No. 60/137,411, filed Jun. 3, 1999, U.S. Ser. No. 60/137,396, filed Jun. 3, 1999, U.S. Ser. No. 60/137,417, filed Jun. 3, 1999, U.S. Ser. No. 60/137,337, filed Jun. 3, 1999, U.S. Ser. No. 60/137,173, filed Jun. 2, 1999, U.S. Ser. No. 60/137,114, filed Jun. 2, 1999, U.S. Ser. No. 60/137,259, filed Jun. 2, 1999, U.S. Ser. No. 60/137,113, filed Jun. 2, 1999, U.S. Ser. No. 60/137,260, filed Jun. 2, 1999, U.S. Ser. No. 60/137,258, filed Jun. 2, 1999, U.S. Ser. No. 60/137,109, filed Jun. 2, 1999, and U.S. Ser. No. 60/137,161, filed Jun. 1, 1999.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (295):

[0294] Many growth factor receptors, including receptors for epidermal growth factor, platelet-derived growth factor, fibroblast growth factor, as well as the growth modulator .alpha.-thrombin, contain intrinsic protein kinase activities. When growth factor binds to the receptor, it triggers the autophosphorylation of a serine, threonine, or tyrosine residue on the receptor. These phosphorylated sites are recognition sites for the binding of other cytoplasmic signaling proteins. These proteins participate in signaling pathways that eventually link the initial receptor activation at the cell surface to the activation of a specific intracellular target molecule. In the case of tyrosine residue autophosphorylation, these signaling proteins contain a common domain referred to as a Src homology (SH) domain. SH2 domains and SH3 domains are found in phospholipase C-.gamma., PI-3-K p85 regulatory subunit, Ras-GTPase activating protein, and pp60.sup.c-src (Lowenstein, E. J. et al. (1992) Cell 70:431-442). The cytokine family of receptors share a different common binding domain and include transmembrane receptors for growth hormone (GH), interleukins, erythropoietin, and prolactin.

Summary of Invention Paragraph - BSTX (754):

[0751] The dithp of the invention may be used for somatic or germline gene therapy. Gene therapy may be performed to (i) correct a genetic deficiency (e.g., in the cases of severe combined immunodeficiency (SCID)-X1 disease characterized by X-linked inheritance (Cavazzana-Calvo, M. et al. (2000) Science 288:669-672), severe combined immunodeficiency syndrome associated with an inherited adenosine deaminase (ADA) deficiency (Blaese, R. M. et al. (1995) Science 270:475-480; Bordignon, C. et al. (1995) Science 270:470-475), cystic fibrosis (Zabner, J. et al. (1993) Cell 75:207-216; Crystal, R. G. et al. (1995) Hum. Gene Therapy 6:643-666; Crystal, R. G. et al. (1995) Hum. Gene Therapy 6:667-703), thalassemias, familial hypercholesterolemia, and hemophilia resulting from Factor VIII or Factor IX deficiencies (Crystal, R. G. (1995) Science 270:404-410; Verma, I. M. and Somia, N. (1997) Nature 389:239-242)), (ii) express a conditionally lethal gene product (e.g., in the case of cancers which result from unregulated cell proliferation), or (iii) express a protein which affords protection against intracellular parasites (e.g., against human retroviruses, such as human immunodeficiency virus (HIV) (Baltimore, D. (1988) Nature 335:395-396; Poeschla, E. et al. (1996) Proc. Natl. Acad. Sci. USA. 93:11395-11399), hepatitis B or C virus (HBV, HCV); fungal parasites, such as Candida albicans and Paracoccidioides brasiliensis; and protozoan parasites such as Plasmodium falciparum and Trypanosoma cruzi). In the case where a genetic deficiency in dithp expression or regulation causes disease, the expression of dithp from an appropriate population of transduced cells may

alleviate the clinical manifestations caused by the genetic deficiency.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040009981 A1

TITLE: Compositions useful as inhibitors of protein kinases

PUBLICATION-DATE: January 15, 2004

INVENTOR-INFORMATION:

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RELATED-US-APPL-DATA:

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US-CL-CURRENT: 514/242, 514/260.1 , 514/263.2 , 514/265.1 , 514/266.23 , 514/269 , 544/182 , 544/262 , 544/277 , 544/280 , 544/284 , 544/317

ABSTRACT:

The present invention relates to compounds useful as inhibitors of protein kinases. The invention also provides pharmaceutically acceptable compositions comprising said compounds and methods of using the compositions in the treatment of various disease, conditions, or disorders.

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Patent Application 60/364,864 filed Mar. 15, 2002 the entirety of which is incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (18):
[0017] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J. 1999, 18, 5019, and Klein et al., Mol. Cell. Biol. 1997, 17, 6427.

Summary of Invention Paragraph - BSTX (165):
[0162] As described generally above, the compounds of the invention are

useful as inhibitors of protein kinases. In one embodiment, the compounds and compositions of the invention are inhibitors of one or more of Aurora-2, GSK-3 or Src kinase, and thus, without wishing to be bound by any particular theory, the compounds and compositions are particularly useful for treating or lessening the severity of a disease, condition, or disorder where activation of one or more of Aurora-2, GSK-3 or Src kinase is implicated in the disease, condition, or disorder. When activation of Aurora-2, GSK-3 or Src is implicated in a particular disease, condition, or disorder, the disease, condition, or disorder may also be referred to as "Aurora-2-, GSK-3-, or Src-mediated disease" or disease symptom. Accordingly, in another aspect, the present invention provides a method for treating or lessening the severity of a disease, condition, or disorder where activation or one or more of Aurora-2, GSK-3 or Src is implicated in the disease state.

Summary of Invention Paragraph - BSTX (166):

[0163] The activity of a compound utilized in this invention as an inhibitor of Aurora-2, GSK-3 or Src kinase, may be assayed in vitro, in vivo or in a cell line. In vitro assays include assays that determine inhibition of either the phosphorylation activity or ATPase activity of activated Aurora-2, GSK-3 or Src kinase. Alternate in vitro assays quantitate the ability of the inhibitor to bind to Aurora-2, GSK-3 or Src kinase. Inhibitor binding may be measured by radiolabelling the inhibitor prior to binding, isolating the inhibitor/Aurora-2, GSK-3 or Src kinase, complex and determining the amount of radiolabel bound. Alternatively, inhibitor binding may be determined by running a competition experiment where new inhibitors are incubated with Aurora-2, GSK-3 or Src kinase bound to known radioligands.

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TITLE: Compositions useful as inhibitors of protein kinases

PUBLICATION-DATE: January 15, 2004

INVENTOR-INFORMATION:

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US-CL-CURRENT: 514/227.8, 514/235.8 , 514/242 , 514/252.19 , 514/269 , 544/123 , 544/182 , 544/295 , 544/317 , 544/60

ABSTRACT:

The present invention relates to compounds useful of inhibitors of protein kinases. The invention also provides pharmaceutically acceptable compositions comprising said compounds and methods of using the compositions in the treatment of various disease, conditions, or disorders.

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Summary of Invention Paragraph - BSTX (20):

[0017] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J. 1999, 18, 5019, and Klein et al., Mol. Cell. Biol. 1997, 17, 6427.

Summary of Invention Paragraph - BSTX (179):

[0174] As described generally above, the compounds of the invention are useful as inhibitors of protein kinases. In one embodiment, the compounds and compositions of the invention are inhibitors of one or more of Aurora-2, GSK-3 or Src kinase, and thus, without wishing to be bound by any particular theory, the compounds and compositions are particularly useful for treating or lessening the severity of a disease, condition, or disorder where activation of one or more of Aurora-2, GSK-3 or Src kinase is implicated in the disease, condition, or disorder. When activation of Aurora-2, GSK-3 or Src is

implicated in a particular disease, condition, or disorder, the disease, condition, or disorder may also be referred to as "Aurora-2-, GSK-3-, or Src-mediated disease" or disease symptom. Accordingly, in another aspect, the present invention provides a method for treating or lessening the severity of a disease, condition, or disorder where activation or one or more of Aurora-2, GSK-3 or Src is implicated in the disease state.

Summary of Invention Paragraph - BSTX (180):

[0175] The activity of a compound utilized in this invention as an inhibitor of Aurora-2, GSK-3 or Src kinase, may be assayed in vitro, in vivo or in a cell line. In vitro assays include assays that determine inhibition of either the phosphorylation activity or ATPase activity of activated Aurora-2, GSK-3 or Src kinase. Alternate in vitro assays quantitate the ability of the inhibitor to bind to Aurora-2, GSK-3 or Src kinase. Inhibitor binding may be measured by radiolabelling the inhibitor prior to binding, isolating the inhibitor/Aurora-2, GSK-3 or Src kinase, complex and determining the amount of radiolabel bound. Alternatively, inhibitor binding may be determined by running a competition experiment where new inhibitors are incubated with Aurora-2, GSK-3 or Src kinase bound to known radioligands.

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TITLE: Compositions useful as inhibitors of protein kinases

PUBLICATION-DATE: January 1, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bebbington, David	Newbury		GB	
Binch, Hayley	Harwell		GB	
Charrier, Jean-Damien	Grove Wantage		GB	
Everitt, Simon	Beaconsfield		GB	
Golec, Julian M. C.	Ashbury		GB	
Kay, David	Wiltshire		GB	
Knegtel, Ronald	Abingdon		GB	
Miller, Andrew	Upton		GB	
Pierard, Francoise	Drayton		GB	

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US-CL-CURRENT: 514/245, 514/227.8 , 514/238.8 , 514/252.19 , 514/275 , 544/113 , 544/122 , 544/198 , 544/209 , 544/295 , 544/324 , 544/60

ABSTRACT:

The present invention relates to compounds useful as inhibitors of protein kinases. The invention also provides pharmaceutically acceptable compositions comprising said compounds and methods of using the compositions in the treatment of various disease, conditions, or disorders.

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Patent Application No. 60/364,840 filed Mar. 15, 2002 the entirety of which is incorporated herein by reference.

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Summary of Invention Paragraph - BSTX (18):

[0017] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J. 1999, 18, 5019, and Klein et

al., Mol. Cell. Biol. 1997, 17, 6427.

Summary of Invention Paragraph - BSTX (169):

[0166] As described generally above, the compounds of the invention are useful as inhibitors of protein kinases. In one embodiment, the compounds and compositions of the invention are inhibitors of one or more of Aurora-2, GSK-3 or Src kinase, and thus, without wishing to be bound by any particular theory, the compounds and compositions are particularly useful for treating or lessening the severity of a disease, condition, or disorder where activation of one or more of Aurora-2, GSK-3 or Src kinase is implicated in the disease, condition, or disorder. When activation of Aurora-2, GSK-3 or Src is implicated in a particular disease, condition, or disorder, the disease, condition, or disorder may also be referred to as "Aurora-2-, GSK-3-, or Src-mediated disease" or disease symptom. Accordingly, in another aspect, the present invention provides a method for treating or lessening the severity of a disease, condition, or disorder where activation or one or more of Aurora-2, GSK-3 or Src is implicated in the disease state.

Summary of Invention Paragraph - BSTX (170):

[0167] The activity of a compound utilized in this invention as an inhibitor of Aurora-2, GSK-3 or Src kinase, may be assayed in vitro, in vivo or in a cell line. In vitro assays include assays that determine inhibition of either the phosphorylation activity or ATPase activity of activated Aurora-2, GSK-3 or Src kinase. Alternate in vitro assays quantitate the ability of the inhibitor to bind to Aurora-2, GSK-3 or Src kinase. Inhibitor binding may be measured by radiolabelling the inhibitor prior to binding, isolating the inhibitor/Aurora-2, GSK-3 or Src kinase, complex and determining the amount of radiolabel bound. Alternatively, inhibitor binding may be determined by running a competition experiment where new inhibitors are incubated with Aurora-2, GSK-3 or Src kinase bound to known radioligands.

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PGPUB-FILING-TYPE: new

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TITLE: Chimeric antigens for eliciting an immune response

PUBLICATION-DATE: January 1, 2004

INVENTOR-INFORMATION:

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DATE FILED: February 13, 2003

RELATED-US-APPL-DATA:

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US-CL-CURRENT: 424/189.1

ABSTRACT:

Disclosed herein are the nucleotide sequences, deduced amino acid sequences as well as methods and compositions necessary to elicit immune responses against chronic Hepatitis B infections in animals and humans. Immune response is enhanced by fusing relevant viral antigens with xenotypic immunoglobulin heavy chain region through a peptide linker and producing the fusion proteins in Baculovirus expression system to incorporate high mannose glycosylation. By virtue of the antibody component, the fusion proteins bind to Fc receptors on the surface of antigen presenting cells, are taken up, processed and derived peptides are presented on MHC Class I, which elicit a CTL (Th1) response. In a similar fashion, due to cross priming and presentation on MHC Class II, will elicit a humoral (Th2) response. In addition, disclosed are the methods of cloning, expression and production of the fusion proteins.

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Summary of Invention Paragraph - BSTX (2):

[0001] The present invention relates to chimeric antigens (fusion proteins) for targeting and activating antigen presenting cells. In particular, the invention describes compositions and methods that contain or use one or more fusion proteins that contain a pre-selected HBV antigen or an HCV antigen, and a xenotypic immunoglobulin fragment, wherein the fusion molecule is capable of binding and activating antigen presenting cells, especially dendritic cells.

Summary of Invention Paragraph - BSTX (4):

[0002] Viral infectious diseases are major public healthcare issues. Human Hepatitis B virus (HBV) is a member of a family of DNA viruses that primarily infect the liver (Gust, 1986). Other members of this family are woodchuck

hepatitis B virus (WHV) ((Summers, Smolec et al. 1978), duck hepatitis B virus (DHBV) ((Mason, Seal et al. 1980) and heron hepatitis B virus (HHBV) (Sprengel, Kaleta et al. 1988). These viruses share a common morphology and replication mechanisms, but are species specific for infectivity (Marion, 1988).

Summary of Invention Paragraph - BSTX (5):

[0003] HBV primarily infects liver cells and can cause acute and chronic liver disease resulting in cirrhosis and hepatocellular carcinoma. Infection occurs through blood and other body fluids. Approximately 90% of the individuals infected by HBV are able to clear the infection, while the remaining 10% become chronic carriers of the virus with a high probability of developing cirrhosis of the liver and hepatocellular carcinoma. The world Health Organization statistics show that more than 2 billion people have been infected by HBV and among these, 350 million are chronically infected by the virus (Beasley 1988) (Law J Y 1993). Prophylactic vaccines based on HBV surface antigen (HbSAg) have been very effective in providing protective immunity against HBV infections. These vaccines have been developed from HbSAg purified from plasma of chronic HBV carriers, produced by recombinant DNA techniques as well as through the use of synthetic peptides (Please see U.S. Pat. Nos. 4,599,230 and 4,599,231). These vaccines are highly effective in the prevention of infection, but are ineffective in eradicating established chronic infections.

Summary of Invention Paragraph - BSTX (6):

[0004] Human Hepatitis B Virus (HBV) belongs to the family of Hepadnaviruses. Other members of this family are Duck Hepatitis B Virus (DHBV), Woodchuk Hepatitis Virus (WHV) Ground squirrel Hepatitis B Virus (GSHV) and Heron Hepatitis B Virus (HHBV). Although these viruses have similar morphology and replication mechanism, they are fairly species specific consequently, infect only very closely related species. These viruses have a DNA genome ranging in size of 3.0-3.2 Kb, with overlapping reading frames to encode several proteins. HBV genome encodes several proteins. Among these, the surface antigens: Large (S1/S2/S), Medium (S2/S) and Small (S) are proposed to be involved in the binding of the virus to the cellular receptors for uptake. The core protein (Core) forms capsids which encapsulate the partially double stranded DNA genome. Polymerase/Reverse Transcriptase (Pol) protein is a multifunctional enzyme necessary for the replication of the virus. The X protein has been proposed to have many properties, including the activation of Src kinases (Ganem and Schneider, 2001). The present invention describes DNA sequences and amino acid compositions of the surface antigen proteins S1/S2, S1/S2/S as well as Core protein fusion proteins with a xenotypic Mab fragment.

Summary of Invention Paragraph - BSTX (16):

[0014] In patients with chronic viral infections, since the virus is actively replicating, viral antigens will be produced within the host cell. Secreted antigens will be present in the circulation. As an example, in the case of chronic HBV carriers, virions, the HBV surface antigens and the core antigens can be detected in the blood. An effective therapeutic vaccine should be able to induce strong CTL responses against an intracellular antigen or an antigen delivered into the appropriate cellular compartment so as to activate the MHC Class I processing pathway.

Summary of Invention Paragraph - BSTX (19):

[0017] Dendritic cells derived from blood monocytes, by virtue of their capability as professional antigen presenting cells have been shown to have great potential as immune modulators which stimulate primary T cell response (Steinman, Inaba et al. 1999), (Banchereau and Steinman 1998). This unique property of the DCs to capture, process, present the antigen and stimulate naive T cells has made them very important tools for therapeutic vaccine

development (Laupeze, Fardel et al. 1999). Targeting of the antigen to the DCs is the crucial step in the antigen presentation and the presence of several receptors on the DCs for the Fc region of monoclonal antibodies have been exploited for this purpose (Regnault, Lankar et al. 1999). Examples of this approach include ovarian cancer Mab-B43.13, Anti-PSA antibody as well as Anti-HBV antibody antigen complexes (Wen, Qu et al. 1999). Cancer immunotherapy using DCs loaded with tumor associated antigens have been shown to produce tumor-specific immune responses and anti-tumor activity (Campton, Ding et al. 2000; Fong and Engleman 2000). Promising results were obtained in clinical trials in vivo using tumor-antigen-pulsed DCs (Tarte and Klein 1999). These studies clearly demonstrate the efficacy of using DCs to generate immune responses against cancer antigens.

Summary of Invention Paragraph - BSTX (23):
[0020] The present invention relates to chronic infectious diseases, and in particular to chronic HBV infections. The presentation of HBV antigens to elicit a CTL response by the use of vaccine molecules designed to target the vaccines to DCs whereby the HBV-associated antigens treated as "self" during the chronic infection will be recognized as "foreign" and the host's immune system will mount a CTL response to eliminate HBV-infected cells. At the same time, through cross presentation, the antibody response to the circulating HBV antigen will bind to the antigen and remove it from the circulation. Accordingly, the present invention is designed to produce vaccines which can induce a broad immune response in patients who have chronic viral infections such as HBV.

Summary of Invention Paragraph - BSTX (24):
[0021] One or more embodiments of the present invention include one or more chimeric antigens suitable for initiating an immune response against Hepatitis B virus (HBV). In these embodiments of the invention, the nucleotide and deduced amino acid sequences for pre-selected HBV antigens are linked to fragments of xenotypic antibodies. The resulting chimeric antigens are capable of targeting and activating antigen presenting cells, such as dendritic cells.

Brief Description of Drawings Paragraph - DRTX (13):
[0038] FIG. 9 shows the nucleotide and deduced amino acid sequences of the expressed HBV S1/S2 protein.

Brief Description of Drawings Paragraph - DRTX (16):
[0041] FIG. 12 shows the nucleotide and deduced amino acid sequences of the expressed HBV S1/S2/S protein.

Brief Description of Drawings Paragraph - DRTX (19):
[0044] FIG. 15 shows the nucleotide and deduced amino acid sequences of the expressed HBV core protein.

Brief Description of Drawings Paragraph - DRTX (37):
[0062] FIG. 33 shows the nucleotide (A) and amino acid (B) sequences of the ORF of HBV S1/S2-TBD in the plasmid pFastbachta-tbd.

Brief Description of Drawings Paragraph - DRTX (38):
[0063] FIG. 34 shows the comparison of binding of HBV S1/S2-TBD, IgG1, and IgG2 over time.

Brief Description of Drawings Paragraph - DRTX (39):

[0064] FIG. 35 shows the comparison of HBV S1/S2-TBD, IgG1, and IgG2a binding to maturing dendritic cells on day 1.

Brief Description of Drawings Paragraph - DRTX (40):

[0065] FIG. 36 shows the comparison of HBV S1/S2-TBD, IgG1, and IgG2a binding to maturing dendritic cells on day 4.

Brief Description of Drawings Paragraph - DRTX (41):

[0066] FIG. 37 shows the comparison of uptake between HBV S1/S2-TBD, IgG1, and IgG2 as a function of concentration.

Brief Description of Drawings Paragraph - DRTX (42):

[0067] FIG. 38 shows the correlation of HBV S1/S2-TBD to CD32 and CD206 expression on dendritic cells.

Brief Description of Drawings Paragraph - DRTX (43):

[0068] FIG. 39 shows that the binding of HBV S1/S2-TBD to DC32 and DC206 receptors on dendritic cells is abolished by anti-Fc Mab.

Detail Description Paragraph - DETX (3):

[0089] In some embodiments of the invention, the IRD of the chimeric antigen includes one or more proteins selected from the group comprising: one or more HBV proteins, one or more recombinant HBV proteins, one or more HCV proteins, or one or more recombinant HCV proteins.

Detail Description Paragraph - DETX (4):

[0090] In yet another embodiment of the invention, IRD of the chimeric antigen includes 6-His-peptide fused to one or more HBV proteins, one or more recombinant HBV proteins, one or more HCV proteins, or one or more recombinant HCV proteins.

Detail Description Paragraph - DETX (32):

[0118] "Immune Response Domain (IRD)" refers to the variously configured antigenic portion of a bifunctional molecule The IRD comprises one or more antigens or one or more recombinant antigens. Viral antigens may include, but are not limited to, HBV PreS1/S2 HBV PreS1/S2/S, HBV Core, HBV Core ctm .alpha.-terminal modified), HBV e-antigen, HBV Polymerase, HCV Core, HCV E1-E2, HCV E1, HCV E2, HCV NS3-serine protease, HCV NS5A and NS4A, HIV GP120 and HSV Alkaline nuclease and HPV Antigens.

Detail Description Paragraph - DETX (41):

[0127] An embodiment of the present invention includes the use of recombinant antigens of HBV, HCV, or DHBV virus fused to a xenotypic antibody fragment by molecular biological techniques, production of the fusion proteins in baculovirus expression system and their use as therapeutic vaccines against chronic HBV and HCV infections. The present invention provides an efficient method to deliver a hitherto unrecognized antigen to APCs in vivo so as to generate a broad immune response, a Th1 response involving CTLs and a Th2 (antibody) response. The immunogenicity of the pre-selected viral antigen unrecognized by the host immune system is increased due to the presence of the xenotypic antibody fragment as well as by the presence of specific glycosylation introduced in the insect cell expression system. The antigen-antibody fragment fusion protein, due to the presence of the antibody

component, will bind to specific receptors present on various immune cell types including dendritic cells, macrophages, B-cells and granulocytes. The fusion proteins administered to either humans or animals will be taken up by the APCs, especially DCs, will be hydrolysed to small peptides and presented on the cell surface, complexed with MHC Class I and/or MHC Class II, which can elicit a broad immune response and clear the viral infection.

Detail Description Paragraph - DETX (42):
[0128] As used herein, the term "Target Binding Domain (TBD)" refers to the region of an immunoglobulin heavy chain constant region, which is a portion capable of binding to an Fc receptor on an APC. This is derived from Mouse anti-HBVsAg Mab (Hybridoma 2C12) as cloned in pFASTBAC HTa expression vector, and expressed in High Five insect cell expression system (Invitrogen). The constant region of the heavy chain of the immunoglobulin molecule consists of part of CH1, and Hinge-CH2-CH3 from N-terminal to C-terminal. The constant region of the IgG1 molecule for the practice of the present invention contains a linker peptide, part of C.sub.H1-hinge and the regions C.sub.H2 and C.sub.H3. The hinge region portion of the monomeric TBD can form disulphide bonds with a second TBD molecule. FIG. 5 illustrates a schematic representation of the TBD molecule. The protein is expressed as an N-terminal fusion protein with 6-Histidine tag, a seven amino acid rTEV protease cleavage site and the N-terminal fusion of the Target Binding Domain (TBD) of the xenotypic (murine) Mab raised against HBV sAg (Hybridoma 2C12). TBD is a fragment of the constant chain of the IgG1 Mab from 2C12 with the sequence of amino acids comprising the 8 amino acid peptide linker, five amino acids of the C.sub.H1 region, the hinge sequences, C.sub.H2 and C.sub.H3 region sequences (FIG. 5). The TBD fragment defined herein forms the parent molecule for the generation of fusion proteins with antigens derived from viruses or other infectious agents. FIG. 1A depicts the formation of dimeric TBD molecule formed via intermolecular disulphide bonds. FIG. 6 shows the nucleotide sequence of the Open Reading Frame (ORF) encoding the TBD protein and the deduced amino acid sequence as defined in FIG. 5.

Detail Description Paragraph - DETX (43):
[0129] FIG. 7 shows a schematic representation of chimeric antigen vaccine molecule, as produced in the insect cell expression system. This molecule is a fusion protein of N-terminal 6-His tag, rTEV protease cleavage site, HBV S1/S2 antigen, linker peptide, a part of the C.sub.H1 as well as C.sub.H2 and C.sub.H3 domains of the mouse monoclonal antibody from 2C12. Cleavage and purification will result in the generation of HBV S1/S2-TBD molecule. FIG. 8 shows the nucleotide and amino acid sequences of the chimeric antigen molecule. FIG. 9 shows the nucleotide and the deduced amino acid sequences of the expressed HBVS1/S2 protein.

Detail Description Paragraph - DETX (44):
[0130] FIG. 10 shows a schematic representation of the fusion protein of HBV S1/S2/S-TBD. FIG. 11 shows the nucleotide and deduced amino acid sequences of the ORF of the fusion protein. FIG. 12 shows the nucleotide and deduced amino acid sequences of the HBV S1/S2/S protein.

Detail Description Paragraph - DETX (45):
[0131] FIG. 13 illustrates the fusion protein of HBV core-TBD molecule as expressed in the insect cells. FIG. 14 shows the nucleotide and amino acid sequences in the ORF of the fusion protein. FIG. 15 shows the nucleotide and deduced amino acid sequences of the HBV Core protein.

Detail Description Paragraph - DETX (46):
[0132] Another embodiment of the present invention involves the production and use of fusion proteins generated from Duck Hepatitis B Virus (DHBV)

antigens and murine TBD. DHBV has been used as a very versatile animal model for the development of therapies for HBV, its human counterpart. DHBV genome encodes Surface antigen PreS/S, Core protein (Core) which form capsids and the polymerase enzyme which serves multiple functions.

Detail Description Paragraph - DETX (62):
[0145] The mouse IgG1 DNA sequences encoding amino acids of CH1-Hinge-CH2-CH3 region was generated form mRNA isolated from the hybridoma (2C12) which produces Mab against HBV surface antigen (sAg). Total mRNA was isolated using TRizol reagent (Gibco BRL cat. No. 15596-026) and the cDNA of the TBD was generated by RT-PCR using Superscript First-strand Synthesis (Invitrogen Cat. No. 11904-018). The PCR primers contained linker sequences encoding the linker peptide --SRPQGGGS-- at the 5' terminus, a unique Not I site at the 5' and a unique Hind III restriction site at the 3' end. The resulting cDNA contains (5' Not I)-linker sequence-CH1 (VDKKI)-CH2-CH3-(3' Hind III). Following digestion with the respective enzymes, the fragment is ligated with pFASTBACHTa expression vector plasmid (Invitrogen) using the same restriction enzyme sites. The 5' primer used for PCR amplification was (Sense) 5' TGTCATTCTGCGGCCGCAAGGCGGCGGGATCCGTGGACAAGAAAATTGTG CCAGG (Seq. ID No. 1) and the 3' primer was (antisense) 5' ACGAATCAAGCTTTGCAGCCCAGGAG- A (Seq. ID No. 2), which contained the Not I and Hind III sites, respectively. The following is the protocol used for directional cloning. The generated fragment was digested with the respective enzymes, purified on agarose gel and cloned into the vector plasmid. The DNA sequence and the correctness of the ORF were verified by standard sequencing methods.

Detail Description Paragraph - DETX (69):
Construction of HBV Surface Antigen S1/S2 and HBV S1/S2-TBD Fusion Protein Expression Vectors

Detail Description Paragraph - DETX (70):
[0151] The DNA encoding the HBV sAg fragment S1/S2 was generated from the plasmid pRSETB HBV S1/S2 template using PCR methodology. The primers used were: (sense) 5' GGATCCTGTACGATGACG (Seq. ID No. 5) and the 3' primer (antisense) 5' AGTCATTCTGCGGCCGCGAGTTCGTCACAGGGTCCCCGG (Seq. ID No. 6) containing the restriction enzyme site Not I. The 5' end contained a unique Bam H I site derived from the parent plasmid which was used for ligations. Amplified DNA was digested with Bam H I/Not I and ligated with pFastBacHTa expression vector to generate the expression plasmid for HBV S1/S2 protein. The fragment was ligated with the plasmid pFastBacHTa-TBD (described in example 1) following the digestion with the respective enzymes. This produced the expression plasmid pFastBacHTa HBV S1/S2-TBD. This plasmid was used to produce recombinant baculovirus (described in example 1) which expressed the chimeric antigen-TBD fusion protein: 6-His tag-rTEV protease cleavage site-HBVS1/S2-TBD (See FIGS. 7-9).

Detail Description Paragraph - DETX (72):
Construction of HBV Surface Antigen S1/S2/S and HBV S1/S2/S-TBD Fusion Protein Expression Vectors

Detail Description Paragraph - DETX (73):
[0152] The DNA encoding the HBV sAg fragment S1/S2/S was generated from the plasmid pALT HBV 991 (University of Alberta) template using PCR methodology. The 5' primer used for the PCR was (sense) 5' GATAAGGATCCTATGGGAGGTTGGTCATCAAAAC (Seq. ID No. 7), containing the restriction enzyme Nco I site. The PCR primer for 3' terminus was (antisense) 5' GTCATACTGCGGCCGCGAAATGTATACCCAGAGACAAAAG (Seq. ID No. 8), containing the restriction enzyme Not I site. Amplified cDNA was digested with the respective enzymes and ligated with pFastBacHTa expression vector to generate either the

expression plasmid for HBV S1/S2/S or the expression plasmid pFastBac HTa HBV S1/S2/S-TBD fusion protein (see FIGS. 10-11).

Detail Description Paragraph - DETX (75):
Construction of HBV Core Antigen and HBV Core-TBD Fusion Protein Expression Vectors

Detail Description Paragraph - DETX (76):
[0153] HBV produces the core proteins (Core) to encapsidate the replicating genome of the virus. There are two forms of the core one secreted into circulation, also known as the "e" antigen and the capsid forming core protein. The present invention also relates to the generation of expression plasmids to produce the Core protein as well as the core antigen-TBD fusion protein, in insect cells. The cDNA encoding the HBV Core protein was generated from the plasmid pALTHBV991 template using PCR technique. The 5' primer used for the PCR was (sense) 5' TGCGCTACCATGGACATTGACCCCTTATAAAG (Seq. ID No. 9) which contains the restriction enzyme Nco I site and the 3' primer used was (antisense) 5' TGTCATTCTGCGGCCGCGAACATTGAGATTCCCGAGATTGAG (Seq. ID No. 10), containing the restriction enzyme Not I site. The PCR-amplified cDNA was digested with the respective enzymes and ligated with pFastBacHTa expression vector to generate either the expression plasmid for HBV Core protein or the expression plasmid pFastBacHTa HBV Core-TBD fusion protein (see FIGS. 13-14).

Detail Description Paragraph - DETX (79):
[0154] DHBV has served as a powerful animal model in the development of antiviral therapy for HBV. Pekin ducks, congenitally infected with DHBV have been used to study-the mechanism of replication of the virus and for the screening of antiviral compounds. The present invention also describes the chimeric DHBV antigen-TBD molecules which could be used as therapeutic vaccines in DHBV-infected ducks, thus providing a viable animal model for the feasibility studies for a HBV therapeutic vaccines.

Detail Description Paragraph - DETX (88):
Chemically Cross-Linked HBV sAg-Fc (Murine)

Detail Description Paragraph - DETX (89):
[0158] HBV sAg was cross linked using the bifunctional cross linking agent DMS, a homobifunctional imidoester which react with amino groups on the proteins. The unreacted components were removed by gel filtration. The conjugate was characterized with respect to the stoichiometry of sAg/Fc in the conjugate and the fraction containing sAg:Fc at 1:1 ratio was chosen for antigen presentation assays using human monocyte-derived immature Dendritic cells (DCs). Immature DCs were cultured for four days with GM-CSF/IL4, incubated with the sAg-Fc conjugate and matured in the presence of TNF.alpha./IFN.alpha.. Autologous CD3+ T cells were added to the mature DCs. Following three rounds of exposure to the mature DCs, T cell stimulation was quantitated by measuring the production of intracellular IFN.gamma., using flow cytometry.

Detail Description Paragraph - DETX (92):
[0161] HBV sAg (US Biologicals; Cat# H 1910-27)

Detail Description Paragraph - DETX (112):
[0179] This protein has been cloned using the pFASTBAC HTa vector and the baculovirus system and expressed in Sf-9 and High Five insect cells, similar to the HBV fusion proteins. This was done as follows. The DNA encoding the HCV core fragment was generated from the plasmid pCV-H77c (NIH) template using PCR methodology.

Detail Description Paragraph - DETX (155):
[0206] To determine the extent of uptake of chimeric antigens (e.g. HBV S1/S2-TBD) compared with IgG1 and IgG2a, cells were incubated with various concentrations of the antigen, IgG1 (2C12, the parent Mab from which TBD was produced) and IgG2a (G155-178) for 1 hour at 37.degree. C. in AIM V media with 0.1% BSA. Cells were washed twice in PBSB and fixed with PBS with 2% PF overnight at 4.degree. C. Subsequently, the cells were washed twice in PBSB and permeablized with PBS containing 0.1% (w/v) saponin (Sigma) for 40 minutes at 20.degree. C.

Detail Description Paragraph - DETX (162):
[0211] The mouse IgG1 DNA sequences encoding amino acids of CH1-Hinge-CH2-CH3 region was generated form mRNA isolated from the hybridoma (2C12) which produces Mab against HBV surface antigen (sAg). Total mRNA was isolated using TRizol reagent (Gibco BRL cat. No. 15596-026) and the cDNA of the TBD was generated by RT-PCR using Superscript First-strand Synthesis (Invitrogen Cat. No. 11904-018). The PCR primers contained linker sequences encoding the linker peptide -SRPQGGGS- (Seq. ID No. _____) at the 5' terminus, a unique Not I site at the 5' and a unique Hind III restriction site at the 3' end. The resulting cDNA contains (5' Not I)-linker sequence-CH1 (VDKKI)-CH2-CH3-(3' Hind III). Following digestion with the respective enzymes, the fragment is ligated with pFASTBACHTa expression vector plasmid (Invitrogen) using the same restriction enzyme sites to generate-pFASTBACHTa-TBD. The 5' primer used for PCR amplification was (Sense) 5' TGTCATTCTGCGGCCGCAAGGCGGCGGGATCCGTGGACAAGAAAATTGTG CCAGG (Seq. ID No. 3) and the 3' primer was (antisense) 5' ACGAATCAAGCTTTGCAGCCCAGGAG- A (Seq. ID No. 4), which contained the Not I and Hind III sites, respectively. The following is the protocol used for directional cloning. The generated fragment was digested with the respective enzymes, purified on agarose gel and cloned into the vector plasmid. The DNA sequence and the correctness of the ORF were verified by standard sequencing methods. Nucleotide sequence of the ORF of TBD in the plasmid pFASTBACHTa-TBD and the deduced amino acid sequences of the expressed TBD protein from the ORF is shown in FIG. 32.

Detail Description Paragraph - DETX (168):
Construction of HBV Surface Antigen S1/S2 and HBV S1/S2-TBD Chimeric Fusion Protein Plasmids

Detail Description Paragraph - DETX (169):
[0214] The DNA encoding the HBV sAg fragment S1/S2 was generated from the plasmid pRSETB HBV S1/S2 template using PCR methodology. The primers used were: (sense) 5' GGATCCTGTACGATGACG (Seq. ID No. 5) and the 3' primer (antisense) 5' AGTCATTCTGCGGCCGCGAGTTCGTCACAGGGTCCCCGG (Seq. ID No. 6) containing the restriction enzyme site Not I. The 5' end contained a unique Bam H I site derived from the parent plasmid which was used for ligations. Amplified DNA was digested with Bam H I/Not I and ligated with pFastBacHTa expression vector to generate the expression plasmid for HBV S1/S2 protein. The fragment was ligated with the plasmid pFastBacHTa-TBD (described in example 3) following the digestion with the respective enzymes. This produced the expression plasmid pFastBacHTa HBV S1/S2-TBD. This plasmid was used to produce recombinant baculovirus (described in example 10) which expressed the chimeric antigen-TBD fusion protein: 6-His tag-rTEV protease cleavage site-HBVS1/S2-TBD. Nucleotide and deduced amino acid sequences from the ORFs of plasmid pFastBacHTa HBV S1/S2 are shown in FIG. 8. Nucleotide and deduced amino acid sequences from the ORFs of plasmid pFastBacHTa HBV S1/S2-TBD are shown in FIG. 33.

Detail Description Paragraph - DETX (171):
Expression and Purification of HBV Surface Antigen S1/S2 and HBVS1/S2-TBD

Chimeric Fusion Proteins

Detail Description Paragraph - DETX (172):
[0215] Recombinant bacmids of standardized multiplicity of infection (MOI) were used to infect High Five insect cells. For suspension cultures, cells were seeded at a density of 3.times.10.sup.5 cells/mL and incubated at 27.5.degree. C. with shaking at 138 rpm until the cell density reached 2-3.times.10.sup.6 cells/mL. Recombinant baculovirus was added to the cells. For the expression of the fusion protein HBV S1/S2-TBD, the MOI was 1 pfu/cell and for S1/S2, 2 pfu/cell were used. The incubation at 27.5.degree. C. was continued for 48 hrs. The cells were harvested by centrifugation at 2,500 rpm for 10 minutes at 4.degree. C. and used for the purification of the recombinant proteins.

Detail Description Paragraph - DETX (173):
[0216] HBV S1/S2 protein and the fusion protein HBV S1/S2-TBD fusion protein were expressed in Express Five Insect cells, purified as described in example 1.b. The proteins were subjected to electrophoresis on a 12% polyacrylamide gel and stained with coomassie blue.

Detail Description Paragraph - DETX (193):
Glycosylation of HBV S1/S2 Antigen Produced in Insect Cells Bind to DCs through CD206 Receptors.

Detail Description Paragraph - DETX (195):
[0226] HBV S1/S2, the antigen component of the chimeric antigen was expressed in both E. coli (no glycosylation) and in High Five insect cells (high mannose glycosylation). These antigens were compared for their binding to DCs. Glycosylated protein showed better binding and uptake by DCs (FIG. 40).

Detail Description Paragraph - DETX (204):
Glycosylation of HBV S1/S2 Antigen Imparts Immunogenicity to the Antigen and Generates Higher T Cell Responses

Detail Description Paragraph - DETX (205):
[0230] Glycosylation of HBV S1/S2 elicits increased immunogenicity and T Cell responses. The insect cell pathway of protein glycosylation is different from that of mammalian cells in that proteins synthesized in insect cells undergo glycosylation that results in high mannose content and a lack of terminal sialic acid residues in the secreted protein (Altman, Staudacher, et al 1999).

Detail Description Paragraph - DETX (206):
[0231] HBV S1/S2, the antigen component of the chimeric antigen was expressed in both E. coli (no glycosylation) and in High Five insect cells (high mannose glycosylation). These antigens were compared for T cell responses when presented by DCs. Both intracellular and secreted IFN.gamma. levels were measured and the results are presented in FIGS. 45 and 46.

Detail Description Paragraph - DETX (208):
Construction of HBV Core Antigen and HBV Core-TBD Fusion Protein Expression Vectors

Detail Description Paragraph - DETX (209):
[0232] HBV produces the core proteins (Core) to encapsidate the replicating genome of the virus. There are two forms of the core one secreted into circulation, also known as the "e" antigen and the capsid forming core protein. The present invention also relates to the generation of expression plasmids to

produce the Core protein as well as the core antigen-TBD fusion protein, in insect cells similar to examples described in example 19. The DNA encoding the HBV Core protein was generated from the plasmid pALTHBV991 template using PCR technique. The 5' primer used for the PCR was (sense) 5' TGCGCTACCATGGACATTGACCCCTTATAAA- G (Seq. ID No. 9) which contains the restriction enzyme Nco I site and the 3' primer used was (antisense) 5' TGTCATTCTGCGGCCGCGAACATTGAGATTCCCGAGATT- GAG (Seq. ID No. 8), containing the restriction enzyme Not I site. The PCR-amplified cDNA was digested with the respective enzymes and ligated with pFastBacHTa expression vector to generate either the expression plasmid for HBV Core protein or the expression plasmid pFastBacHTa HBV Core-TBD fusion protein. Nucleotide and deduced amino acid sequences from the ORFs of plasmid pFastBacHTa HBV core are shown in FIG. 15. Nucleotide and deduced amino acid sequences from the ORFs of plasmid pFastBacHTa HBV Core-TBD are shown in FIG. 14.

Detail Description Paragraph - DETX (212):
[0233] DHBV has served as a powerful animal model in the development of antiviral therapy for HBV. Pekin ducks, congenitally infected with DHBV have been used to study the mechanism of replication of the virus and for the screening of antiviral compounds. The present invention also describes the chimeric DHBV antigen-TBD molecules which could be used as therapeutic vaccines in DHBV-infected ducks, thus providing a viable animal model for the feasibility studies for a HBV therapeutic vaccines.

Detail Description Paragraph - DETX (253):
[0258] 7. Chimeric antigens include fusion proteins from HBV surface antigens (S1/S2), HBV Core and TBD, derived from the murine Mab 2C12.

Detail Description Paragraph - DETX (258):
[0263] 12. Chimeric antigen fusion protein HBV surface antigen S1/S2-TBD and HBV surface antigen S1/S2 has been purified.

Detail Description Paragraph - DETX (263):
[0268] 17. Chimeric antigen fusion protein HBV surface antigen S1/S2-TBD enhances the antigen presentation by professional antigen presenting cells (DCs).

Detail Description Paragraph - DETX (264):
[0269] 18. DCs loaded with the Chimeric antigen fusion protein HBV surface antigen S1/S2-TBD, on presentation to T cells elicit an immune response.

Detail Description Paragraph - DETX (286):
[0291] Description of Artificial Sequence: Seq. ID No. 5: HBV S1/S2 5' PCR amplification primer

Detail Description Paragraph - DETX (291):
[0296] Description of Artificial Sequence: Seq. ID No.6: HBV S1/S2 3' PCR amplification primer

Detail Description Paragraph - DETX (296):
[0301] Description of Artificial Sequence: Seq. ID No. 7: HBV S1/S2/S 5' PCR Amplification Primer

Detail Description Paragraph - DETX (301):
[0306] Description of Artificial Sequence: Seq. ID No. 8: HBV S1/S2/S 3' PCR Amplification Primer

Claims Text - CLTX (3):
2. The composition of claim 1 wherein the immune response domain comprises

one or more proteins and recombinant proteins selected from the group consisting of one or more HBV proteins and recombinant proteins thereof, and one or more DHBV proteins and recombinant proteins thereof, and one or more HCV proteins and recombinant proteins thereof.

Claims Text - CLTX (4):
3. The composition of claim 2 wherein the immune response domain comprises one or more of HBV proteins and recombinant proteins selected from a group consisting of HBV S1/S2, HBV S1/S2/S, HBV Core, HBV Core ctm and HBV polymerase.

Claims Text - CLTX (21):
20. A composition for eliciting a T-cell response in vivo comprising contacting an antigen presenting cell with a chimeric antigen comprising an immune response domain and a target binding domain, wherein the immune response domain comprises one or more sequences comprising an HBV core protein, HBV S protein, HBV S1 protein, HBV S2 protein, combinations thereof, and recombinant molecules thereof, and wherein the target binding domain comprises a xenotypic antibody fragment.

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ABSTRACT:

The present invention provides compositions and methods for utilizing recombinant alphavirus vectors. Also disclosed are compositions and methods for making and utilizing eukaryotic layered vector initiation systems.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of co-pending PCT application US94/10469, filed Sep. 15, 1994. In addition, this application is a

continuation-in-part of pending U.S. patent application Ser. No. 08/376,184, filed Jan. 18, 1995, which is a continuation-in-part of pending U.S. patent application Ser. No. 08/348,472, filed Nov. 30, 1994, which is a continuation-in-part of pending U.S. patent application Ser. No. 08/198,450, filed Feb. 18, 1994, which was a continuation-in-part of pending U.S. patent application Ser. No.08/122,791, filed Sep. 15, 1993.

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Summary of Invention Paragraph - BSTX (24):
[0022] In still other embodiments, the vectors described above include a selected heterologous sequence which may be obtained from a virus selected from the group consisting of influenza virus, HPV, HBV, HCV, EBV, HIV, HSV, FeLV, FIV, Hanta virus, HTLV I, HTLV II and CMV. Within one preferred embodiment, the heterologous sequence obtained from HPV encodes a protein selected from the group consisting of E5, E6, E7 and L1. In yet other embodiments, the vectors described above include a selected heterologous sequence encoding an HIV protein selected from the group consisting of HIV gp120 and gag.

Summary of Invention Paragraph - BSTX (25):
[0023] The selected heterologous sequences described above also may be an antisense sequence, noncoding sense sequence, or ribozyme sequence. In preferred embodiments, the antisense or noncoding sense sequence is selected from the group consisting of sequences which are complementary to influenza virus, HPV, HBV, HCV, EBV, HV, HSV, FeLV, FIV, Hanta virus, HTLV I, HTLV II, and CMV sequences.

Summary of Invention Paragraph - BSTX (37):
[0035] Within another aspect, HBV-derived and coronavirus-derived packaging cell lines are provided which are suitable for packaging and production of and alphavirus vector. Within one embodiment, an HBV-derived packaging cell line is provided, comprising an expression cassette which directs the expression of HBV core, preS/S, and P proteins. Within another embodiment, a coronavirus-derived packaging cell line is provided, comprising an expression cassette which directs the expression of coronavirus N, M, and S proteins.

Detail Description Paragraph - DETX (61):
[0137] Within related aspects of the present invention, alphavirus cell-specific expression vectors may be constructed to express viral antigens, ribozyme, antisense sequences or immunostimulatory factors such as gamma-interferon (.gamma.-IFN), IL-2 or IL-5 for the targeted treatment of virus infected cell types. In particular, in order to target alphavirus vectors to specific foreign organism or pathogen-infected cells, inverted repeats of the alphavirus vector may be selected to hybridize to any pathogen-specific RNA, for instance target cells infected by pathogens such as HIV, CMV, HBV, HPV and HSV.

Detail Description Paragraph - DETX (72):
[0148] Another example of an immunomodulatory cofactor is the, B7/BB1 costimulatory factor. Briefly, activation of the full functional activity of T cells requires two signals. One signal is provided by interaction of the antigen-specific T cell receptor with peptides which are bound to major histocompatibility complex (MHC) molecules, and the second signal, referred to as costimulation, is delivered to the T cell by antigen-presenting cells. Briefly, the second signal is required for interleukin-2 (IL-2) production by T cells and appears to involve interaction of the B7/BB1 molecule on antigen-presenting cells with CD28 and CTLA-4 receptors on T lymphocytes (Linsley et al., J. Exp. Med., 173:721-730, 1991a, and J. Exp. Med.,

174:561-570, 1991). Within one embodiment of the invention, B7/BB1 may be introduced into tumor cells in order to cause costimulation of CD8.sup.+T cells, such that the CD8.sup.+T cells produce enough IL-2 to expand and become fully activated. These CD8.sup.+T cells can kill tumor cells that are not expressing B7 because costimulation is no longer required for further CTL function. Vectors that express both the costimulatory B7/BB1 factor and, for example, an immunogenic HBV core protein, may be made utilizing methods which are described herein. Cells transduced with these vectors will become more effective antigen-presenting cells. The HBV core-specific CTL response will be augmented from the fully activated CD8.sup.+T cell via the costimulatory ligand B7/BB1.

Detail Description Paragraph - DETX (109):

[0185] Within other aspects of the present invention, alphavirus vector constructs are provided which direct the expression of immunogenic portions of antigens from foreign organisms or other pathogens. Representative examples of such antigens include bacterial antigens (e.g., E. coli, streptococcal, staphylococcal, mycobacterial, etc.), fungal antigens, parasitic antigens, and viral antigens (e.g., influenza virus, Human Immunodeficiency Virus ("HIV"), Hepatitis A, B and C Virus ("HAV", "HBV" and "HCV", respectively), Human Papiloma Virus ("HPV"), Epstein-Barr Virus ("EBV"), Herpes Simplex Virus ("HSV"), Hantavirus, TTLV 1, HTLV II and Cytomegalovirus ("CMV"). As utilized within the context of the present invention, "immunogenic portion" refers to a portion of the respective antigen which is capable, under the appropriate conditions, of causing an immune response (i.e., cell-mediated or humoral). "Portions" may be of variable size, but are preferably at least 9 amino acids long, and may include the entire antigen. Cell-mediated immune responses may be mediated through Major Histocompatability Complex ("MHC") class I presentation, MHC Class II presentation, or both.

Detail Description Paragraph - DETX (110):

[0186] Within one aspect of the invention, alphavirus vector constructs are provided which direct the expression of immunogenic portions of Hepatitis B antigens. Briefly, the Hepatitis B genome is comprised of circular DNA of about 3.2 kilobases in length and has been well characterized (Tiollais et al., Science 213:406-411, 1981; Tiollais et al., Nature 317:489-495, 1985; and Ganem and Varmus, Ann. Rev. Biochem. 56:651-693, 1987; see also EP 0 278,940, EP 0 241,021, WO 88/10301, and U.S. Pat. Nos. 4,696,898 and 5,024,938, which are hereby incorporated by reference). The Hepatitis B virus presents several different antigens, including among others, three HB "S" antigens (HBsAgs), an HBc antigen (HBcAg), an HBe antigen (HBeAg), and an HBx antigen (HBxAg) (see Blum et al., TIG 5(5):154-158, 1989). Briefly, the HBeAg results from proteolytic cleavage of a P22 pre-core intermediate and is secreted from the cell. HBeAg is found in serum as a 17 kD protein. The HBcAg is a protein of 183 amino acids, and the HBxAg is a protein of 145 to 154 amino acids, depending on subtype.

Detail Description Paragraph - DETX (112):

[0188] As will be evident to one of ordinary skill in the art, various immunogenic portions of the above-described S antigens may be combined in order to induce an immune response when administered by one of the alphavirus vector constructs described herein. In addition, due to the large immunological variability that is found in different geographic regions for the S open reading frame of HBV, particular combinations of antigens may be preferred for administration in particular geographic regions. Briefly, epitopes that are found in all human hepatitis B virus S samples are defined as determinant "a". Mutually exclusive subtype determinants; however, have also been identified by two-dimensional double immunodiffusion (Ouchterlony, Progr. Allergy 5:1, 1958). These determinants have been designated "d" or "y" and "w" "or" "r"

(LeBouvier, J. Infect. 123:671, 1971; Bancroft et al., J. Immunol. 109:842, 1972; and Courouce et al., Bibl. Haematol. 42:1-158, 1976). The immunological variability is due to single nucleotide substitutions in two areas of the hepatitis B virus S open reading frame, resulting in the following amino acid changes: (1) exchange of lysine-122 to arginine in the Hepatitis B virus S open reading frame causes a subtype shift from d to y, and (2) exchange of arginine-160 to lysine causes the shift from subtype r to w. In Africans, subtype ayw is predominant, whereas in the U.S. and northern Europe the subtype adw.sub.2 is more abundant (Molecular Biology of the Hepatitis B Virus, McLachlan (ed.), CRC Press, 1991). As will be evident to one of ordinary skill in the art, it is generally preferred to construct a vector for administration which is appropriate to the particular hepatitis B virus subtype which is prevalent in the geographical region of administration. Subtypes of a particular region may be determined by two-dimensional double immunodiffusion or, preferably, by sequencing the S open reading frame of HBV virus isolated from individuals within that region.

Detail Description Paragraph - DETX (113):

[0189] Also presented by HBV are pol ("HBV pol"), ORF 5, and ORF 6 antigens. Briefly, the polymerase open reading frame of HBV encodes reverse transcriptase activity found in virions and core-like particles in infected livers. The polymerase protein consists of at least two domains: the amino terminal domain which encodes the protein that primes reverse transcription, and the carboxyl terminal domain which encodes reverse transcriptase and RNase H activity. Immunogenic portions of HBV pol may be determined utilizing methods described herein (e.g., below and in Example 13), utilizing alphavirus vector constructs described below, and administered in order to generate an immune response within a warm-blooded animal. Similarly, other HBV antigens, such as ORF 5 and ORF 6 (Miller et al., Hepatology 9:322-327, 1989) may be expressed utilizing alphavirus vector constructs as described herein. Representative examples of alphavirus vector constructs utilizing ORF 5 and ORF 6 are set forth below in the examples.

Detail Description Paragraph - DETX (129):

[0205] A wide variety of vector systems may be utilized as the first layer of the eukaryotic layered vector initiation system, including for example, viral vector constructs developed from DNA viruses such as those classified in the Poxviridae, including for example canary pox virus or vaccinia virus (e.g., Fisher-Hoch et al., PNAS 86:317-321, 1989; Flexner et al., Ann. N.Y. Acad. Sci. 569:86-103, 1989; Flexner et al., Vaccine 8:17-21, 1990; U.S. Pat. Nos. 4,603,112, 4,769,330 and 5,017,487; WO 89/01973); Papoviridae such as BKV, JCV or SV40 (e.g., Mulligan et al., Nature 277:108-114, 1979); Adenoviridae, such as adenovirus (e.g., Berkner, Biotechniques 6:616-627, 1988; Rosenfeld et al., Science 252:431-434, 1991); Parvoviridae, such as adeno-associated virus (e.g., Samulski et al., J. Vir. 63:3822-3828, 1989; Mendelson et al., Virol. 166:154-165, 1988; PA 7/222,684); Herpesviridae, such as Herpes Simplex Virus (e.g., Kit, Adv. Exp. Med. Biol. 215:219-236, 1989); and Hepadnaviridae (e.g., HBV), as well as certain RNA viruses which replicate through a DNA intermediate, such as the Retroviridae (see, e.g., U.S. Pat. No. 4,777,127, GB 2,200,651, EP 0,345,242 and W091/02805; Retroviridae include leukemia in viruses such as MoMLV and immunodeficiency viruses such as HIV, e.g., Poznansky, J. Virol. 65:532-536, 1991).

Detail Description Paragraph - DETX (149):

[0225] Within other aspects of the present invention, compositions and methods are provided for administering an alphavirus vector construct which is capable of preventing, inhibiting, stabilizing or reversing infectious, cancerous, auto-immune or immune diseases. Representative examples of such diseases include viral infections such as HIV, HBV HTLV I, HTLV II, CMV, EBV

and HPV, melanomas, diabetes, graft vs. host disease, Alzheimer's disease and heart disease.

Detail Description Paragraph - DETX (453):

[0525] Using techniques described above, the lacZ gene encoding the .beta.-galactosidase reporter protein was cut from the plasmid pSV-.beta.-galactosidase (PROMEGA CORP, Madison, Wis.) and substituted into the ELVIS-luc plasmid DNA vector in place of luciferase. To examine in vivo gene expression from ELVIS vectors, Balb/c mice and rats are injected intramuscularly (i.m.) with ELVIS-.beta.-gal or ELVIS-luc plasmid DNA vectors. FIG. 24 demonstrates the in vivo expression of .beta.-galactocidase in muscle tissue taken from a rat and stained with X-gal at three days post i.m. injection. Mice injected with ELVIS-.beta.-gal also demonstrate positively staining blue muscle fibers. Luciferase expression levels from muscle which were between 75- and 300-fold higher than control levels were detected in 3/4 Balb/c mice at two days post i.m. inoculation with ELVIS-luc plasmid. In other experiments, C3H/HeN mice were injected i.m. with ELVIS vectors expressing either the hepatitis B virus core (HBV-core) or hepatitis B virus e (HBV-e) proteins. Using ELISA detection systems, both HBV-core- and HBV-e-specific IgG antibodies were detected in serum samples collected from the mice 10 days following the second injection with the vectors. These experiments demonstrate that Sindbis-derived DNA vectors are able to express foreign genes in vivo, in rat and mouse muscle.

Detail Description Paragraph - DETX (565):

[0633] In the derivation of alphavirus vector packaging and producer cell lines, many approaches are outlined to control the expression of viral genes, such that producer cell lines stably transformed with both vector and vector packaging cassettes, can be derived. These approaches include inducible and/or cellular differentiation sensitive promoters, antisense structural genes, heterologous control systems, and mosquito or other cells in which viral persistent infections are established. Regardless of the final configuration for the alphavirus vector producer cell line, the ability to establish persistent infection, or at least delay cell death as a result of viral gene expression, may be enhanced by inhibiting apoptosis. For example, the DNA tumor viruses, including adenovirus, HPV, SV40, and mouse polyomavirus (Py), transform cells in part, by binding to, and inactivating, the retinoblastoma (Rb) gene product p105 and its closely related gene product, p107, and other gene products involved in the control of the cell cycle including cyclin A, p33.sup.cdk2 and p34.sup.cdc2. All of these viruses, except for Py, encode gene products which bind to and inactivate p53. Uniquely, Py encodes middle T antigen (mT) which binds to and activates the membrane tyrosine kinase, src, and also phosphatidylinositol-3-kinase, which is required for the full transformation potential of this virus (Talmage et al., Cell 59:55-65, 1989). The binding to and inactivation of the Rb and p53 recessive oncogene products prevents cells transformed by these DNA tumor viruses from entering the apoptotic pathway. It is known that p53 is able to halt the division of cells, in part by inhibiting the expression of proteins associated with cellular proliferation, including c-fos, hsc70, and bcl-2 (Miyashita et al., Cancer Research 54:3131-3135,1994).

Detail Description Paragraph - DETX (676):

[0742] In another embodiment, a RNA packaging signal derived from another virus is inserted into the alphavirus vector to allow packaging by the structural proteins of that corresponding virus. For example, the 137 nt. packaging signal from hepatitis B virus, located between nts. 3134 and 88 and spanning the precore/core junction (Junker-Niepmann et al. EMBO J. 9:3389, 1990), is amplified from an HBV template using two oligonucleotide primers. PCR is performed using a standard three temperature cycling protocol, plasmid

pHBV1.1 (Junker-Niepmann et al. EMBO J. 9:3389, 1990) as the template, and the following oligonucleotide pair, each of which contain 20 nucleotides complementary to the HBV sequence and flanking ApaI recognition sequences:

Detail Description Paragraph - DETX (677):
[0743] Following amplification, the PCR amplicon is digested with ApaI and purified from a 1.5% agarose gel using MERMAID.TM. (Bio101). Sindbis vector plasmid pKSSINdlJRsjrc (Example 3) also is digested with ApaI, under limited conditions to cleave at only one of its two sites, followed by treatment with CIAP, purification from a 1% agarose gel, and ligation with the above-synthesized HBV amplicon, to produce a construct designated pKSSINhbvJR. Other alphavirus vectors (see Example 3) are readily modified in a similar manner. Cell lines which express the HBV core, preS/S, and P proteins necessary for packaging of the RNA sequence are derived by modification of helper plasmid pCH3143 (Junker-Niepmann et al., EMBO J. 9:3389, 1990) to include a selectable marker. An expression cassette containing the neomycin resistance marker is obtained by digestion of plasmid pBK-RSV (Stratagene) with Mst II and blunt-ending with Klenow fragment. The selectable marker is then ligated into any one of several unique sites within pCH3143 that have been digested and their termini made blunt. The resulting construct is transfected into a desired cell line, for example, mouse hepatoma line Hepal-6 (ATCC #CRL1830), and selected using the drug G418, as described in Example 7. Introduction of the pKSSINhbvJR vector, or related RNA- or DNA-based alphavirus vectors, results in the production of packaged vector particles with the same hepatotropism as HBV.

Detail Description Paragraph - DETX (679):
[0745] Following amplification, the PCR amplicon is digested with ApaI, purified from a 1.5% agarose gel using MERMAID.TM., and ligated into pKSSINdlJRsjrc, prepared as described for HBV. The resulting construct is designated pKSSINmhvJR. Other alphavirus vectors (see Example 3) are readily. modified in a similar manner. Packaging of vectors modified with this MHV sequence is accomplished by using expression cassettes which produce each of the required coronavirus structural proteins: nucleocapsid (N protein; Armstrong et al., NAR 11:883, 1983); membrane (M protein, Armstrong et al., Nature 308:751, 1984); and spike (S protein, Luytjes et al., Virology 161:479, 1987). Preferably, these proteins are inserted into the vector-inducible pVGELVSdl-linker plasmid (described previously in this example) and selected for with the G418 drug following transfection into the appropriate cell type. Other expression methodologies (see Example 7) may also be readily utilized. Addtional coronaviruses, for example, human coronaviruses OC43 (ATCC #VR-759) and 229E (ATCC #VR-740), can be readily used in place of MHV to produce packaged recombinant alphavirus particles which show tropism for cells in the respiratory tract.

Detail Description Paragraph - DETX (682):
[0748] In each case, the packaging sequences from HBV, coronavirus, retrovirus, or any other virus, also may be incorporated into alphavirus vectors at locations other than those outlined above, provided the location is not present in the subgenomic transcript. For example, the next most preferable site of insertion is the carboxy-terminal region of nonstructural protein 3, which has been shown to be highly variable in both length and sequence among all alphaviruses for which sequence information is available. Further, these applications are not limited by the ability to derive the corresponding packaging cell lines, as the necessary structural proteins also may be expressed using any of the altenative approaches described in Example 8.

Detail Description Paragraph - DETX (717):
[0779] Generation of Vector Constructs which Express HBV Antigens for the

Induction of an Immune Response

Detail Description Paragraph - DETX (718):
[0780] A. Isolation of HBV E/Core Sequence

Detail Description Paragraph - DETX (721):
[0783] 1. Site-Directed Mutagenesis fo HBV E/Core Sequence Utilizing PCR

Detail Description Paragraph - DETX (729):
[0791] The second primer corresponds to the anti-sense nucleotide sequence from SK+ plasmid polylinker and contains a Cla I site 135 bp downstream of the stop codon of the HBV precore/core coding region.

Detail Description Paragraph - DETX (733):
[0795] The second primer sequence corresponds to the anti-sense nucleotide sequence from the SK+ plasmid polylinker and contains a Cla I site 135 bp downstream of the stop codon of the HBV precore/core coding region.

Detail Description Paragraph - DETX (735):
[0797] The first PCR reaction corrects the deletion in the antisense strand and the second reaction corrects the deletion in the sense strands. PCR reactions one and two correct the mutation from CC to CCA which occurs in codon 79 and a base pair substitution from TCA to TCT in codon 81. Primer 1 contains two consecutive Xho I sites 10 bp upstream of the ATG codon of HBV e coding region and primer 4 contains a Cla I site 135 bp downstream of the stop codon of HBV precore/core coding region. The products of the first and second PCR reactions are extended in a third PCR reaction to generate one complete HBV precore/core coding region with the correct sequence.

Detail Description Paragraph - DETX (738):
[0800] 2. Isolation of HBV Core Sequence

Detail Description Paragraph - DETX (752):
[0814] The PCR product from the third reaction yields the correct sequence for HBV precore/core coding region.

Detail Description Paragraph - DETX (753):
[0815] To isolate HBV core coding region, a primer is designed to introduce the Xho I restriction site upstream of the ATG start codon of the core coding region, and eliminate the 29 amino acid leader sequence of the HBV precore coding region. In a fourth reaction, the HBV core coding region is produced using the PCR product from the third reaction and the following two primers.

Detail Description Paragraph - DETX (756):
[0818] The second primer corresponds to the anti-sense nucleotide sequence for the T-3 promoter present in the SK+HBe plasmid. The approximately 600 bp PCR product from the fourth PCR reaction contains the HBV core coding region and novel Xho I restriction sites at the 5' end and Cla I restriction sites at the 3' end that was present in the multicloning site of SK+HBe plasmid.

Detail Description Paragraph - DETX (758):
[0820] Following the fourth PCR reaction, the solution is transferred into a fresh 1.5 ml microfuge tube. Fifty microliters of 3 M sodium acetate is added to this solution followed by 500 .mu.l of chloroform:isoamyl alcohol (24:1). The rnixture is vortexed and then centrifuged at 14,000 rpm for 5 minutes. The aqueous phase is transferred to a fresh microfuge tube and 1.0 ml 100% EtOH is added. This solution is incubated at -20.degree. C. for 4.5 hours, and then centrifuged at 10,000 rpm for 20 minutes. The supernatant is decanted, and the pellet rinsed with 500 .mu.l of 70% EtOH. The pellet is dried by

centrifugation at 10,000 rpm under vacuum and then resuspended in 10 .mu.l deionized H.sub.2O. One microliter of the PCR product is analyzed by 1,.5% agarose gel electrophoresis. The approximately 600 bp Xho I-Cla I HBV core PCR fragment is cloned into the Xho I-Cla I site of SK+ plasmid. This plasmid is designated SK+HBc.

Detail Description Paragraph - DETX (759):
[0821] 3. Isolation of HBV X Antigen

Detail Description Paragraph - DETX (762):
[0824] 4. Constructions of Sindbis Vectors Expressing HBVE HBV Core and HBV X

Detail Description Paragraph - DETX (764):
[0826] Construction of a Sindbis vector expressing the HBV core sequence is accomplished by digestion of plasmid SK+HBc (described above) with Xho I and Xba I. The HBc fragment is isolated by agarose gel electrophoresis, purified by GENECLEAN.TM. and ligated into pKSSINBV at the Xho I and Xba I sites. This Sindbis-HBc vector is designated pKSSIN-HBc.

Detail Description Paragraph - DETX (765):
[0827] Construction of a Sindbis vector expressing the HBV-X antigen sequence is accomplished by digesting the plasmid SK-X Ag with Xho I and Xba I to release a cDNA fragment encoding HBV-X sequences. The fragment is isolated by agarose gel electrophoresis, purified using GENECLEAN.TM., and inserted into pKSSINBV, pre-treated with Xho I and Xba I enzymes. This Sindbis-HBx vector is designated pKSIN-HBx.

Detail Description Paragraph - DETX (766):
[0828] The above Sindbis HBV expressing vectors may also be modified to coexpress a selectable drug resistance marker dependent on the requirements of the experiment or treatment of the vector infected cells. In particular, any of the above Sindbis HBV expression vectors described may also be designed to coexpress G418 resistance. This is accomplished by incorporating an internal ribosomal entry site (Example 5) followed by the bacterial neomycin phosphotransferase gene placed 3' of the HBV coding sequences and 5' of the terminal 3' end of the vector using the multiple cloning site of the vector. These G418 resistant vector constructs can be used for selecting vector infected cells for the generation of HBV specific CTL targets in the following sections.

Detail Description Paragraph - DETX (769):
[0831] Cell lysates from cells infected by any of the HBV expressing vectors are made by washing 1.0.times.10.sup.7 cultured cells with PBS, resuspending the cells to a total volume of 600 .mu.l in PBS, and sonicating for two 5-second periods at a setting of 30 in a Branson sonicator, Model 350 (Fisher, Pittsburgh, Pa.) or by freeze thawing three times. Lysates are clarified by centrifugation at 10,000 rpm for 5 minutes.

Detail Description Paragraph - DETX (771):
[0833] As shown in FIG. 16, using these procedures approximately 100-200 ng/ml HBV e antigen is expressed in the cell lysates and 300-400 ng/ml HBV e antigen is secreted from BHK cells infected with the Sin BV HB e vector.

Detail Description Paragraph - DETX (772):
[0834] As shown in FIG. 17, using these procedures, approximately 40 ng/ml HBV core antigen is expressed in the cell lysates, from 10.sup.6 BHK cells infected with the Sin BV HBcore. Mouse fibroblast cells infected with the recombinant HBcore Sindbis vector express 6-7 fold higher HBV core protein

levels than the recombinant HBcore retroviral vector transduced cells (WO 93/15207). As shown in FIG. 18, using these procedures, approximately 12-14 ng/ml HBV core antigen is expressed in the cell lysates from 10.sup.6 L-M(TK-) cells infected with the SinBVHBcore vector as compared to the approximately 2 ng/ml HBV core antigen expressed from recombinant HBcore retroviral vector transducer cells.

Detail Description Paragraph - DETX (780):
[0842] Using these procedures, it can be shown that CTLs to HBV e antigen can be induced.

Detail Description Paragraph - DETX (784):
[0846] Lymphoblastoid cell lines (LCL) are established for each patient by infecting (transforming) their B-cells with fresh Epstein-Barr virus (EBV) taken from the supematant of a 3-week-old culture of B95-8, EBV transformed marmoset leukocytes (ATCC CRL 1612). Three weeks after EBV-transformation, the LCL are infected with Sindbis vector expressing HBV core or e antigen and G418 resistance. Vector infection of LCL is accomplished by infecting LCL cells with packaged alphavirus vector particles produced from the appropriaste cell line The culture medium consists of RPMI 1640, 20% heat inactivated fetal bovine serum (Hyclone, Logan, Utah), 5.0 mM sodium pyruvate and 5.0 mM non-essential amino acids. Infected LCL cells are selected by adding 800 .mu.g/ml G418. The Jurkat A2/K.sup.b cells (L. Sherman, Scripps Institute, San Diego, Calif.) are infected essentially as described for the infection of LCL cells.

Detail Description Paragraph - DETX (788):
[0850] Humoral immune responses in mice specific for HBV core and e antigens are detected by ELISA. The ELISA protocol utilizes 100 .mu.g/well of recombinant HBV core and recombinant HBV e antigen (Biogen, Geneva, Switzerland) to coat 96-well plates. Sera from mice immunized with vector expressing HBV core or HBV e antigen are then serially diluted in the antigen-coated wells and incubated for 1 to 2 hours at room temperature. After incubation, a mixture of rabbit anti-mouse IgGl, IgG2a, IgG2b, and IgG3 with equivalent titers is added to the wells. Horseradish peroxidase ("HRP")-conjugated goat anti-rabbit anti-serum is added to each well and the samples are incubated for 1 to 2 hours at room temperature. After incubation, reactivity is visualized by adding the appropriate substrate. Color will develop in wells that contain IgG antibodies specific for HBV core or HBV e antigen.

Detail Description Paragraph - DETX (790):
[0852] Antigen induced T-helper activity resulting from two or three injections of Sindbis vector expressing HBV core or e antigen, is measured in vitro. Specifically, splenocytes from immunized mice are restimulated in vitro at a predetermined ratio with cells expressing HBV core or e antigen or with cells not expressing HBV core or e antigen as a negative control. After five days at 37.degree. C. and 5% CO.sub.2 in RPMI 1640 culture medium containing 5% FBS, 1.0 mM sodium pyruvate and 10.sup.-5 2-mercaptoethanol, the supernatant is tested for IL-2 activity. IL-2 is secreted specifically by T-helper cells stimulated by HBV core or e antigen, and its activity is measured using the CTL clone, CTLL-2 (ATCC TIB 214). Briefly, the CTLL-2 clone is dependent on IL-2 for growth and will not proliferate in the absence of IL-2. CTLL-2 cells are added to serial dilutions of supernatant test samples in a 96-well plate and incubated at 37.degree. C. and 5%, CO.sub.2 for 3 days. Subsequently, 0.5 .mu.l Ci .sup.3H-thymidine is added to the CTLL-2 cells. 0.5Ci .sup.3H-thymidine is incorporated only if the CTLL-2 cells proliferate. After an overnight incubation, cells are harvested using a PHD cell harvester (Cambridge Technology Inc., Watertown, Mass.) and counted in a Beckman beta

counter. The amount of IL-2 in a sample is determined from a standard curve generated from a recombinant IL-2 standard obtained from Boehringer Mannheim (Indianapolis, Ind.).

Detail Description Paragraph - DETX (794):
[0856] The mouse system may also be used to evaluate the induction of humoral and cell-mediated immune responses with direct administration of Sindbis vector encoding HBV core or e antigen. Briefly, six- to eight-week-old female C3H/He mice are injected intramuscularly (i.m.) with 0.1 ml of reconstituted (with sterile deionized, distilled water) or intraperitoneally (ip) with 1.0 ml of lyophilized HBV core or HBV e expressing Sindbis vector. Two injections are given,one week apart. Seven days after the second injection, the animals are sacrificed. Chromium release CTL assays are then performed essentially as described in Example 13E 1.a.

Detail Description Paragraph - DETX (796):
[0858] The data generated in the mouse system described above is used to determine the protocol of administration of vector in chimpanzees chronically infected with hepatitis B virus. Based on the induction of HBV-specific CILs in mice, the subjects in chimpanzee trials will receive four doses of vector encoding core or e antigen at 7 day intervals given in two successively escalating dosage groups. Control subjects will receive a placebo comprised of formulation media. The dosage will be either 10.sup.7 or 10.sup.8 pfu given in four 1.0 ml injections i.m. on each injection day. Blood samples will be drawn on days 0, 14, 28, 42, 56, 70, and 84 in order to measure serum alanine aminotransferase (ALT) levels, the presence of hepatitis B e antigen, the presence of antibodies directed against the hepatitis B e antigen, serum HBV DNA levels and to assess safety and tolerability of the treatment. The hepatitis B e antigen and antibodies to HB e antigen is detected by Abbott HB e rDNA EIA kit (Abbott Laboratories Diagnostic Division, Chicago, Ill.) and the serum HBV DNA levels is determined by the Chiron bDNA assay. Efficacy of the induction of CTLs against hepatitis B core or e antigen can be determined as in Example 13E 1.c.

Detail Description Paragraph - DETX (797):
[0859] Based on the safety and efficacy results from the chimpanzee studies, the dosage and inoculation schedule is determined for administration of the vector to subjects in human trials. These subjects are monitored for serum ALT levels, presence of HBV e antigen, the presence of antibodies directed against the HBV e antigen and serum HBV DNA levels essentially as described above. Induction of human CTLs against hepatitis B core or e antigen is determined as in Example 13E 1.c.

Detail Description Paragraph - DETX (798):
[0860] G. Generation of ELVIS Vector Constructs which Express HBV Antigens for the Induction of an Immune Response

Detail Description Paragraph - DETX (799):
[0861] 1. Construction of ELVIS Vectors Expressing HBVE-C, HBV Core and HBV X

Detail Description Paragraph - DETX (800):
[0862] Construction of an ELVIS vector expressing the HBV e antigen is accomplished by digesting the SK+HB e-c plasmid with Xho I and Not I to release the cDNA fragment encoding HBVe-c sequences. The fragment is then isolated by agarose gel electrophoresis, purified using GENECLEAN.TM., and inserted into pVGELVIS-SINBV-linker vector, previously prepared by digestion with Xho I and Not I. This construct is designated pVGELVIS-HBe.

Detail Description Paragraph - DETX (801):
[0863] The HBcore PCR product described previously is digested with Xho I and Cla I, isolated by agarose gel electrophoresis, purified using GENECLEAN.TM., and ligated into SK+II (Bluescript, Stratagene, Calif.) digested with Xho I and Cla 1. This construct is designated SK+HBcore. Construction of the ELVIS vector expressing the HBV core sequence is accomplished by digesting the SK+HBcore plasmid with Xho I and Not I to release the cDNA fragment encoding HBVcore sequences. The fragment is then isolated by agarose gel electrophoresis, purified using GENECLEAN.TM., and inserted into pVGELVIS-SINBV-linker vector, prepared by digestion with Xho I and Not I. This construct is designated pVGELVIS-HBcore.

Detail Description Paragraph - DETX (802):
[0864] Construction of the ELVIS vector expressing the HBV-X antigen sequence is accomplished by digesting the plasmid SK-X Ag with Xho I and Not I to release the cDNA fragment encoding HBV-X sequences. The fragment is then isolated by agarose gel electrophoresis, purified using GENECLEAN, and inserted into the pVGELVIS-SINBV-linker vector, prepared by digestion with Xho I and Not I. This construct is designated pVGELVIS-HBX.

Detail Description Paragraph - DETX (803):
[0865] Any of the above three constructs can be used for selecting vector infected cells for the generation of HBV specific CTL targets in the following sections.

Detail Description Paragraph - DETX (805):
[0867] The pVGELVIS-HBe plasmid DNA is isolated and purified, and 2 ug of pVGELVIS-HBe DNA is complexed with 10 ul of LIPOFECTMINE.TM. and transfected into 2.times.10.sup.5 BHK cells contained in 35 mM petri plates. Two days post-transfection, supernatants and whole cell lysates were collected and an ELISA assay (see below) was used to determine the amount of expressed HBV-E antigen.

Detail Description Paragraph - DETX (808):
[0870] As shown in FIG. 19, using these procedures, approximately 2 ng/ml HBV e antigen is expressed in the cell lysates and also secreted from BHK cells transfected with different clones of the pVGELVISHBe plasmid.

Detail Description Paragraph - DETX (812):
[0874] The mouse model system is also used to evaluate the induction of humoral and cell-mediated immune responses following direct administration of ELVIS vector expressing HBV core or e antigen. Briefly, six- to eight-week-old female Balb/c, C57B 1/6, C3H/He mice (Charles River, Mass,) and HLA A2.1 transgenic mice (V. Engelhard, Charlottesville, Va.) are injected intramuscularly (i.m.) with, for example, 50 ug or greater, pVGELVIS-HBcore, pVGELVIS-HBVe or pVGELVIS-HBX vector DNA. Two injections are given one week apart. Seven or fourteen days after the second injection, the animals are sacrificed. Chromium release CTL assays are then performed essentially as described in Example 13E 1.a. Detection of humoral immune responses in mice is performed essentialy as described in Example 13E 2 and detection of T cell proliferation in mice is performed essentially as described in Example 13E 3.

Claims Text - CLTX (11):
10. The eukaryotic layered vector initiation system according to either of claims 1 or 2, wherein said selected heterologous sequence is obtained from a virus selected from the group consisting of influenza virus, respiratory syncytial virus, HPV, HBV, HCV, EBV, HIV, HSV, FeLV, FIV, Hanta virus, HTLV I, HTLV II, and CMV.

Claims Text - CLTX (13):

12. The eukaryotic layered vector initiation system according to either of claims 1 or 2, wherein said antisense or non-coding sense sequence is selected from the group consisting of sequences which are complementary to influenza virus, respiratory syncytial virus, HPV, HBV, HCV, EBV, HIV, HSV, and CMV sequences.

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ABSTRACT:

The present invention relates, in general, to an extracellular signal regulated kinase, ERK-5. In particular, the present invention relates to nucleic acid molecules coding for ERK-5; ERK-5 polypeptides; recombinant nucleic acid molecules; cells containing the recombinant nucleic acid molecules; antisense ERK-5 nucleic acid constructs; antibodies having binding affinity to an ERK-5 polypeptide; hybridomes containing the antibodies; nucleic acid probes for the detecting of ERK-5 nucleic acid; a method of detecting ERK-5 nucleic acid or polypeptide in a sample; kits containing nucleic acid probes or antibodies; a method of detecting a compound capable of binding to ERK-5 or a fragment thereof; a method of detecting an agonist or antagonist of ERK-5 activity; a method of agonizing or antagonizing ERK-5 associated activity in a mammal; a method of treating diabetes mellitus, skeletal muscle diseases, Alzheimer's

disease, or peripheral neuropathies in a mammal with an agonist or antagonist of ERK-5 activity; and a pharmaceutical composition comprising an ERK-5 agonist or antagonist.

RELATED APPLICATIONS

[0001] The present application is a continuation-in-part of allowed U.S. application Ser. No. 08/029,404, filed Mar. 19, 1993, which is incorporated herein by reference in its entirety, including any drawings.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (13):

[0012] While some of these muscle specific transcription factors, namely MyoD and myf5, are constitutively expressed both in cycling myoblasts as well as in myotubes, myogenin expression is induced when myoblasts start to differentiate. However, not only transcriptional regulation, but also posttranslational modification such as phosphorylation which has been reported for MyoD1 and myogenin as well as myf5 may influence commitment to myogenesis and maintenance of the differentiated state. Activated oncogenes like ras and src as well as growth factors which are involved in or initiate signal transduction, inhibit myogenesis. In addition, PKC is able to phosphorylate myogenin and could be a major mediator of this inhibition.

Detail Description Paragraph - DETX (216):

[0256] NIH3T3 cells, immortalized mouse fibroblasts (Jainchill et al., J. Virol. 4:549-553 (1969)) were grown in DMEM with 4.5 mg/ml glucose and 10% FCS to subconfluency and transfected with 20 .mu.g/1.times.10.sup.7 cells of a cvn-construct containing the complete hERK-5 cDNA. The cvn vector carries the SV40 early promoter, HBV poly A signal as well as a neomycin resistance gene which allows selection of transfected cells on G418 resistance, and the gene for the DHFR which can be used to increase the expression of the integrated cDNA by addition of methotrexate at concentrations of 100-1000 nM to the culture medium (Rosenthal et al., Cell 46:155-169 (1986)). Transfection was performed as described in Example 4. After 16 h at 35.degree. C. and 3% CO.sub.2, the medium was changed and the cells were grown at 37.degree. C., 5% CO.sub.2 for additional 24 h with one medium change after 8 h. The cells were then split to different dilutions and grown in 1 mg/ml G418 containing medium until cell colonies appeared which were isolated and selected on methotrexate growth.

PGPUB-DOCUMENT-NUMBER: 20030225073

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030225073 A1

TITLE: Compositions useful as inhibitors of protein kinases

PUBLICATION-DATE: December 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Pierce, Albert C.	Cambridge		US	

APPL-NO: 10/ 389707

DATE FILED: March 14, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60364842 20020315 US

US-CL-CURRENT: 514/227.8, 514/235.8 , 514/241 , 514/242 , 544/112 , 544/113 , 544/182 , 544/209 , 544/60

ABSTRACT:

The present invention relates to compounds useful as inhibitors of protein kinases. The invention also provides pharmaceutically acceptable compositions comprising said compounds and methods of using the compositions in the treatment of various disease, conditions, or disorders.

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Patent Application No. 60/364,842 filed Mar. 15, 2002 the entirety of which is incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (18):
[0017] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J. 1999, 18, 5019, and Klein et al., Mol. Cell. Biol. 1997, 17, 6427.

Summary of Invention Paragraph - BSTX (180):
[0177] As described generally above, the compounds of the invention are

useful as inhibitors of protein kinases. In one embodiment, the compounds and compositions of the invention are inhibitors of one or more of Aurora-2, GSK-3 or Src kinase, and thus, without wishing to be bound by any particular theory, the compounds and compositions are particularly useful for treating or lessening the severity of a disease, condition, or disorder where activation of one or more of Aurora-2, GSK-3 or Src kinase is implicated in the disease, condition, or disorder. When activation of Aurora-2, GSK-3 or Src is implicated in a particular disease, condition, or disorder, the disease, condition, or disorder may also be referred to as "Aurora-2, GSK-3, or Src-mediated disease" or disease symptom. Accordingly, in another aspect, the present invention provides a method for treating or lessening the severity of a disease, condition, or disorder where activation or one or more of Aurora-2, GSK-3 or Src is implicated in the disease state.

Summary of Invention Paragraph - BSTX (181):

[0178] The activity of a compound utilized in this invention as an inhibitor of Aurora-2, GSK-3 or Src kinase, may be assayed in vitro, in vivo or in a cell line. In vitro assays include assays that determine inhibition of either the phosphorylation activity or ATPase activity of activated Aurora-2, GSK-3 or Src kinase. Alternate in vitro assays quantitate the ability of the inhibitor to bind to Aurora-2, GSK-3 or Src kinase. Inhibitor binding may be measured by radiolabelling the inhibitor prior to binding, isolating the inhibitor/Aurora-2, GSK-3 or Src kinase, complex and determining the amount of radiolabel bound. Alternatively, inhibitor binding may be determined by running a competition experiment where new inhibitors are incubated with Aurora-2, GSK-3 or Src kinase bound to known radioligands.

PGPUB-DOCUMENT-NUMBER: 20030207873

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030207873 A1

TITLE: Inhibitors of Src and other protein kinases

PUBLICATION-DATE: November 6, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Harrington, Edmund	South Boston	MA	US	

APPL-NO: 10/ 119890

DATE FILED: April 10, 2002

US-CL-CURRENT: 514/227.8, 514/235.8 , 514/241 , 514/252.19 , 514/275 , 544/122 , 544/212 , 544/295 , 544/331 , 544/60

ABSTRACT:

The present invention provides compounds of formula I: 1 wherein A is N or CR, and R.sup.1, G, and R.sup.2, are as described in the specification. These compounds are inhibitors of protein kinase, particularly inhibitors of Src mammalian protein kinase involved in cell proliferation, cell death and response to extracellular stimuli. The invention also relates to methods for producing these inhibitors. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Patent Application 60/282,935 filed Apr. 10, 2001, the contents of which are incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (9):
[0008] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J., 18, 5019, (1999) and Klein et al., Mol. Cell. Biol., 17, 6427 (1997).

Summary of Invention Paragraph - BSTX (22):
[0021] Accordingly, there is still a great need to develop potent inhibitors of JNK3, Src, and Lck protein kinases that are useful in treating various diseases or conditions associated with JNK3, Src, and Lck activation.

Summary of Invention Paragraph - BSTX (78):
[0075] The activity of a compound utilized in this invention as an inhibitor of JNK3, Lck, or Src, may be assayed in vitro, in vivo or in a cell line according to methods known in the art. In vitro assays include assays that determine inhibition of either the phosphorylation activity or ATPase activity

of activated JNK3, Lck, or Src. Alternate in vitro assays quantitate the ability of the inhibitor to bind to JNK3, Lck, or Src. Inhibitor binding may be measured by radiolabelling the inhibitor prior to binding, isolating the inhibitor/JNK3, inhibitor/Lck, or inhibitor/Src complex and determining the amount of radiolabel bound. Alternatively, inhibitor binding may be determined by running a competition experiment where new inhibitors are incubated with JNK3, Lck, or Src bound to known radioligands. Detailed conditions for assaying a compound utilized in this invention as an inhibitor of JNK3, Lck, or Src kinase are set forth in the Examples below.

PGPUB-DOCUMENT-NUMBER: 20030198975

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030198975 A1

TITLE: Proteins associated with cell growth, differentiation, and death

PUBLICATION-DATE: October 23, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 287218

DATE FILED: October 31, 2002

RELATED-US-APPL-DATA:

child 10287218 A1 20021031

parent continuation-of PCT/US02/11152 20020405 US PENDING

non-provisional-of-provisional 60349705 20020115 US

non-provisional-of-provisional 60295263 20010601 US

non-provisional-of-provisional 60295340 20010601 US

non-provisional-of-provisional 60293727 20010525 US

non-provisional-of-provisional 60291846 20010518 US

non-provisional-of-provisional 60291662 20010516 US

non-provisional-of-provisional 60287228 20010427 US

non-provisional-of-provisional 60286820 20010426 US

non-provisional-of-provisional 60283294 20010411 US

non-provisional-of-provisional 60282110 20010406 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
WO	PCT/US02/11152	2002WO-PCT/US02/11152	April 5, 2002

US-CL-CURRENT: 435/6, 435/183 , 435/320.1 , 435/325 , 435/69.1 , 514/44 , 530/350 , 530/388.26 , 536/23.2

ABSTRACT:

The invention provides human proteins associated with cell growth, differentiation, and death (CGDD) and polynucleotides which identify and encode CGDD. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of CGDD.

[0001] This application is a continuation application of PCT application PCT/US02/11152, filed in English on Apr. 5, 2002 and which will be published in English, which claims the benefit of provisional applications U.S. Ser. No. 60/282,110, filed Apr. 6, 2001, U.S. Ser. No. 60/283,294, filed Apr. 11, 2001, U.S. Ser. No. 60/286,820, filed Apr. 26, 2001, U.S. Ser. No. 60/287,228, filed Apr. 27, 2001, U.S. Ser. No. 60/291,662, filed May 16, 2001, U.S. Ser. No. 60/291,846, filed May 18, 2001, U.S. Ser. No. 60/293,727, filed May 25, 2001, U.S. Ser. No. 60/295,340, filed Jun. 1, 2001, U.S. Ser. No. 60/295,263, filed Jun. 1, 2001, and U.S. Ser. No. 60/349,705, filed Jan. 15, 2002, all of which applications and patents are hereby incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (120):

[0119] Ras regulates other signaling pathways by direct interaction with different cellular targets (Katz, M. E. and McCormick, F. (1997) Curr. Opin. Genet. Dev. 7:75-79). One such target is RalGDS, a guanine nucleotide dissociation stimulator for the Ras-like GTPase, Ral (Albright, C. F. et al. (1993) EMBO J. 12:339-347). RalGDS couples the Ras and Ral signaling pathways. Epidermal growth factor (EGF) stimulates the association of RalGDS with Ras in mammalian cells, which activates the GEF activity of RalGDS (Kikuchi, A. and Williams, L. T. (1996) J. Biol. Chem. 271:588-594; Urano, T. et al. (1996) EMBO J. 15:810-816). Ral activation by Ral-GDS leads to activation of Src, a

tyrosine kinase that phosphorylates other molecules including transcription factors and components of the actin cytoskeleton (Goi, T. et al. (2000) EMBO J. 19:623-630). Ral interacts with a number of signaling molecules including Ral-binding protein, a GAP for the Rho-like GTPases; Cdc42 and Rac, which regulate cytoskeletal rearrangement; and phospholipase D1, which is involved in vesicular trafficking (Feig, L. A. et al. (1996) Trends Biochem. Sci. 21:438-441; Voss, M. et al. (1999) J. Biol. Chem. 274:34691-34698).

Summary of Invention Paragraph - BSTX (359):

[0355] In another embodiment of the invention, polynucleotides encoding CGDD may be used for somatic or germline gene therapy. Gene therapy may be performed to (i) correct a genetic deficiency (e.g., in the cases of severe combined immunodeficiency (SCID)-X1 disease characterized by X-linked inheritance (Cavazzana-Calvo, M. et al. (2000) Science 288:669-672), severe combined immunodeficiency syndrome associated with an inherited adenosine deaminase (ADA) deficiency (Blaese, R. M. et al. (1995) Science 270:475-480; Bordignon, C. et al. (1995) Science 270:470-475), cystic fibrosis (Zabner, J. et al. (1993) Cell 75:207-216; Crystal, R. G. et al. (1995) Hum. Gene Therapy 6:643-666; Crystal, R. G. et al. (1995) Hum. Gene Therapy 6:667-703), thalassamias, familial hypercholesterolemia, and hemophilia resulting from Factor VIII or Factor IX deficiencies (Crystal, R. G. (1995) Science 270:404-410; Verma, I. M. and N. Somia (1997) Nature 389:239-242)), (ii) express a conditionally lethal gene product (e.g., in the case of cancers which result from unregulated cell proliferation), or (iii) express a protein which affords protection against intracellular parasites (e.g., against human retroviruses, such as human immunodeficiency virus (HIV) (Baltimore, D. (1988) Nature 335:395-396; Poeschla, E. et al. (1996) Proc. Natl. Acad. Sci. USA 93:11395-11399), hepatitis B or C virus (HBV, HCV); fungal parasites, such as Candida albicans and Paracoccidioides brasiliensis; and protozoan parasites such as Plasmodium falciparum and Trypanosoma cruzi). In the case where a genetic deficiency in CGDD expression or regulation causes disease, the expression of CGDD from an appropriate population of transduced cells may alleviate the clinical manifestations caused by the genetic deficiency.

PGPUB-DOCUMENT-NUMBER: 20030171389

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030171389 A1

TITLE: Inhibitors of Src and Lck protein kinases

PUBLICATION-DATE: September 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bemis, Guy	Arlington	MA	US	
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Harrington, Edmund	South Boston	MA	US	
Salituro, Francesco	Marlboro	MA	US	
Wang, Jian	Boston	MA	US	
Ledeboer, Mark	Acton	MA	US	

APPL-NO: 10/ 171895

DATE FILED: June 14, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60302969 20010703 US

US-CL-CURRENT: 514/275, 544/331

ABSTRACT:

The present invention provides compounds of formula I: 1
or a pharmaceutically acceptable derivative thereof, wherein A-B is N--O or O--N and G, R.sup.1, R.sup.2, R.sup.3, and R.sup.4 are as described in the specification. These compounds are inhibitors of protein kinase, particularly inhibitors of Src and Lck kinase. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Patent Application No. 60/302,969 filed Jul. 3, 2001, the contents of which are incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (9):
[0008] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J., 18, 5019, (1999) and Klein et al., Mol.Cell. Biol., 17, 6427 (1997).

Summary of Invention Paragraph - BSTX (12):
[0011] There is a high unmet medical need to develop new therapeutic agents that are useful in treating the aforementioned conditions associated with Src

and Lck kinase activation, especially considering the currently available, relatively inadequate treatment options for the majority of these conditions.

Summary of Invention Paragraph - BSTX (102):

[0099] The activity of a compound utilized in this invention as an inhibitor of Lck or Src protein kinase may be assayed in vitro, in vivo or in a cell line according to methods known in the art. In vitro assays include assays that determine inhibition of either the phosphorylation activity or ATPase activity of activated Lck or Src. Alternate in vitro assays quantitate the ability of the inhibitor to bind to Lck or Src. Inhibitor binding may be measured by radiolabelling the inhibitor prior to binding, isolating the inhibitor/Lck or inhibitor/Src complex and determining the amount of radiolabel bound. Alternatively, inhibitor binding may be determined by running a competition experiment where new inhibitors are incubated with Lck or Src bound to known radioligands. Detailed conditions for assaying a compound utilized in this invention as an inhibitor of Lck or Src kinase are set forth in the Examples below.

PGPUB-DOCUMENT-NUMBER: 20030144309

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030144309 A1

TITLE: Inhibitors of Src and other protein kinases

PUBLICATION-DATE: July 31, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Choon-Moon, Young	Lexington	MA	US	

APPL-NO: 10/ 146984

DATE FILED: May 16, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60291340 20010516 US

US-CL-CURRENT: 514/275, 514/227.8 , 514/235.8 , 514/252.19 , 514/253.09 , 514/341 , 544/122 , 544/124 , 544/295 , 544/331 , 544/360 , 544/60 , 546/275.4

ABSTRACT:

The present invention provides compounds of formula I: 1 wherein A is N or CR, and G, R.sup.1, R.sup.2 and R.sup.3 are as described in the specification. These compounds are inhibitors of protein kinase, particularly inhibitors of Src mammalian protein kinase involved in cell proliferation, cell death and response to extracellular stimuli. The invention also relates to methods for producing these inhibitors. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application 60/291,340 filed May 16, 2001, the contents of which are incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (9):
[0008] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J., 18, 5019, (1999) and Klein et al., Mol.Cell. Biol., 17, 6427 (1997).

Summary of Invention Paragraph - BSTX (22):
[0021] Accordingly, there is still a great need to develop potent inhibitors of JNK3, Src, and Lck protein kinases that are useful in treating various diseases or conditions associated with JNK3, Src, and Lck activation.

Detail Description Paragraph - DETX (54):

[0077] The activity of a compound utilized in this invention as an inhibitor of JNK3, Lck, or Src, may be assayed in vitro, in vivo or in a cell line according to methods known in the art. In vitro assays include assays that determine inhibition of either the phosphorylation activity or ATPase activity of activated JNK3, Lck, or Src. Alternate in vitro assays quantitate the ability of the inhibitor to bind to JNK3, Lck, or Src. Inhibitor binding may be measured by radiolabelling the inhibitor prior to binding, isolating the inhibitor/JNK3, inhibitor/Lck, or inhibitor/Src complex and determining the amount of radiolabel bound. Alternatively, inhibitor binding may be determined by running a competition experiment where new inhibitors are incubated with JNK3, Lck, or Src bound to known radioligands. Detailed conditions for assaying a compound utilized in this invention as an inhibitor of JNK3, Lck, or Src kinase are set forth in the Examples below.

US-PAT-NO: 6689778

DOCUMENT-IDENTIFIER: US 6689778 B2

TITLE: Inhibitors of Src and Lck protein kinases

DATE-ISSUED: February 10, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bemis; Guy	Arlington	MA	N/A	N/A
Gao; Huai	Natick	MA	N/A	N/A
Harrington; Edmund	South Boston	MA	N/A	N/A
Salituro; Francesco	Marlboro	MA	N/A	N/A
Wang; Jian	Boston	MA	N/A	N/A
Ledeboer; Mark	Acton	MA	N/A	N/A

APPL-NO: 10/ 171895

DATE FILED: June 14, 2002

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Patent Application No. 60/302,969 filed Jul. 3, 2001, the contents of which are incorporated herein by reference.

US-CL-CURRENT: 514/235.8, 514/252.19 , 514/275 , 544/122 , 544/331

ABSTRACT:

The present invention provides compounds of formula I: ##STR1##

or a pharmaceutically acceptable derivative thereof, wherein A--B is N--O or O--N and G, R.sup.1, R.sup.2, R.sup.3, and R.sup.4 are as described in the specification. These compounds are inhibitors of protein kinase, particularly inhibitors of Src and Lck kinase. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

17 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (9):

Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor **HBx activates Src** in a step required for propagation of the virus. Klein et al., EMBO J., 18, 5019, (1999) and Klein et al., Mol.Cell. Biol., 17, 6427 (1997).

Brief Summary Text - BSTX (12):

There is a high unmet medical need to develop new therapeutic agents that are useful in treating the aforementioned conditions associated with Src and Lck kinase activation, especially considering the currently available, relatively inadequate treatment options for the majority of these conditions.

Brief Summary Text - BSTX (77):

The activity of a compound utilized in this invention as an inhibitor of Lck or Src protein kinase may be assayed in vitro, in vivo or in a cell line according to methods known in the art. In vitro assays include assays that determine inhibition of either the phosphorylation activity or ATPase activity of activated Lck or Src. Alternate in vitro assays quantitate the ability of the inhibitor to bind to Lck or Src. Inhibitor binding may be measured by radiolabelling the inhibitor prior to binding, isolating the inhibitor/Lck or inhibitor/Src complex and determining the amount of radiolabel bound. Alternatively, inhibitor binding may be determined by running a competition experiment where new inhibitors are incubated with Lck or Src bound to known radioligands. Detailed conditions for assaying a compound utilized in this invention as an inhibitor of Lck or Src kinase are set forth in the Examples below.

US-PAT-NO: 6642227

DOCUMENT-IDENTIFIER: US 6642227 B2

TITLE: Inhibitors of c-Jun N-terminal kinases (JNK) and other protein kinases

DATE-ISSUED: November 4, 2003

INVENTOR-INFORMATION:

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Moon; Young-Choon	Lexington	MA	N/A	N/A
Wang; Jian	Boston	MA	N/A	N/A
Ledeboer; Mark	Acton	MA	N/A	N/A
Harrington; Edmund	South Boston	MA	N/A	N/A
Gao; Huai	Natick	MA	N/A	N/A

APPL-NO: 10/ 121035

DATE FILED: April 10, 2002

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims benefit of priority to U.S. provisional applications 60/283,621 filed Apr. 13, 2001, 60/329,440 filed Oct. 15, 2001 and 60/292,974 filed May 23, 2001.

US-CL-CURRENT: 514/227.8, 514/235.8 , 514/252.19 , 514/275 , 544/122 , 544/331 , 544/60

ABSTRACT:

The present invention provides compounds of formula I: ##STR1##

or a pharmaceutically acceptable derivative o thereof, wherein A, B, R.sup.a, R.sup.1, R.sup.2, R.sup.3, and R.sup.4 are as described in the specification. These compounds are inhibitors of protein kinase, particularly inhibitors of JNK, a mammalian protein kinase involved cell proliferation, cell death and response to extracellular stimuli; Lck and Src kinase. The invention also relates to methods for producing these inhibitors. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

31 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (19):
Src also plays a role in the replication of hepatitis B virus. The virally

encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J., 18, 5019, (1999) and Klein et al., Mol. Cell. Biol., 17, 6427 (1997).

Brief Summary Text - BSTX (22):

Accordingly, there is still a great need to develop potent inhibitors of JNKs and Src family kinases that are useful in treating various conditions associated with JNK and Src activation.

Brief Summary Text - BSTX (137):

The activity of the compounds of this invention as kinase inhibitors may be assayed in vitro, in vivo or in a cell line. In vitro assays include assays that determine inhibition of either the kinase activity or ATPase activity of activated enzyme, for example JNK, Lck, or Src. Alternate in vitro assays quantitate the ability of the inhibitor to bind to JNK, Lck, or Src and may be measured either by radiolabelling the inhibitor prior to binding, isolating the inhibitor/JNK, inhibitor/Lck, or inhibitor/Src complex and determining the amount of radiolabel bound, or by running a competition experiment where new compounds are incubated with JNK, Lck, or Src bound to known radioligands. One may use any type or isoform of JNK, Lck, or Src, depending upon which JNK, Lck, or Src type or isoform is to be inhibited. The details of the conditions used for the enzymatic assays are set forth in the Examples hereinbelow.

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	174	hbx	US-PGPUB; USPAT	OR	OFF	2004/09/21 09:05
L2	492	(hepatitis adj b adj virus or hbv) near4 inhibit$8	US-PGPUB; USPAT	OR	OFF	2004/09/21 09:07
L3	26	1 and 2	US-PGPUB; USPAT	OR	OFF	2004/09/21 09:07
L4	11	hbx near4 inhibit$8	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:02
L5	10160	src	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:08
L6	794441	activat$8	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:08
L7	239593	upstream	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:09
L8	3912	6 near5 7	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:09
L9	27	8 same 5	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:13
L10	97	5 same 6 same 7	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:13
L11	334	5 near2 (activator$1 or activation)	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:20
L12	4205	hbv or hbx	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:20
L13	19	11 and 12	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:26
L14	798	5 near5 6	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:27
L15	55	12 and 14	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:27
L16	11548	cyclosporin or csa	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:48
L17	2	16 same 12	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:49
L18	417	bapta or cgp37157	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:52
L19	6	18 and 12	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:52

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TITLE: Preventives and remedies for chronic hepatitis

PUBLICATION-DATE: September 11, 2003

INVENTOR-INFORMATION:

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JP	11/374087	1999JP-11/374087	December 28, 1999

PCT-DATA:
APPL-NO: PCT/JP00/09393
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PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 514/12

ABSTRACT:

To provide a prophylactic or therapeutic agent for chronic hepatitis, capable of inhibiting the binding of hepatitis virus and a target cell, thereby preventing or treating chronic hepatitis; and a fusion interferon capable of still more improving a viral clearance ratio at the termination of interferon administration. A prophylactic or therapeutic agent for chronic hepatitis, comprising an oligopeptide having the following characteristics: (A) having a binding affinity to a viral antigen protein; (B) inhibiting binding of virus to a receptor protein on a target cell for said virus; and (C) having homologies with said receptor protein at the amino acid sequence level; a fusion interferon containing an amino acid sequence of the above-mentioned oligopeptide; and method for treating chronic hepatitis in combination with the use of interferon, characterized by using the oligopeptide or a part thereof in combination with interferon.

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Summary of Invention Paragraph - BSTX (7):
[0005] On the other hand, as a method of treating chronic type-B hepatitis, an interferon treatment method is carried out as in the case of chronic type-C hepatitis; however, the therapeutic effect can be expected currently on the

level of only 20% or so. Recently, in the treatment of chronic type-B hepatitis, an inhibitor for an HBV polymerase has been administered; however, the administration has the large side effects and relapse of virus is found after the termination of administration. Further, upon the development of fulminant type-B hepatitis, a method for treatment in combination with the use of interferon and cyclosporin is carried out after exchange of whole serum; however, a lifesaving rate is low currently. As described above, a treatment method which is truly effective for chronic type-B hepatitis and chronic type-C hepatitis which may lead to development of cirrhosis or hepatocellular carcinoma has not been established currently, even though hepatitis B virus and hepatitis C virus have been distributed world-widely and virus carriers are present at 1% or so or greater of the population.

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	174	hbx	US-PGPUB; USPAT	OR	OFF	2004/09/21 09:05
L2	492	(hepatitis adj b adj virus or hbv) near4 inhibit$8	US-PGPUB; USPAT	OR	OFF	2004/09/21 09:07
L3	26	1 and 2	US-PGPUB; USPAT	OR	OFF	2004/09/21 09:07
L4	11	hbx near4 inhibit$8	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:02
L5	10160	src	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:08
L6	794441	activat$8	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:08
L7	239593	upstream	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:09
L8	3912	6 near5 7	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:09
L9	27	8 same 5	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:13
L10	97	5 same 6 same 7	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:13
L11	334	5 near2 (activator$1 or activation)	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:20
L12	4205	hbv or hbx	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:20
L13	19	11 and 12	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:26
L14	798	5 near5 6	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:27
L15	55	12 and 14	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:27
L16	11548	cyclosporin or csa	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:48
L17	2	16 same 12	US-PGPUB; USPAT	OR	OFF	2004/09/21 10:49

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TITLE: Keratinocytes expressing exogenous angiogenic growth factors

PUBLICATION-DATE: January 15, 2004

INVENTOR-INFORMATION:

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RELATED-US-APPL-DATA:

non-provisional-of-provisional 60376488 20020430 US

US-CL-CURRENT: 435/366

ABSTRACT:

The present invention relates to in vitro cultured skin tissue, and in particular to cultured skin tissue comprising exogenous genes encoding angiogenic growth factors. In some embodiments, the keratinocytes express exogenous angiopoietin-1 or a member of the VEGF family, preferably VEGF-A. In particularly preferred embodiments, the keratinocytes are incorporated into cultured skin tissue.

[0001] This application claims the benefit of provisional application 60/376,488, filed Apr. 30, 2002.

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Detail Description Paragraph - DETX (50):

[0066] The NIKS cell line is useful for in vitro assays because it provides a consistent source of genetically identical human keratinocytes. The NIKS cells arose from the BC-1-Ep strain of human neonatal foreskin keratinocytes isolated from an apparently normal male infant. In early passages, the BC-1-Ep cells exhibited no morphological or growth characteristics that were atypical for cultured normal human keratinocytes. Cultivated BC-1-Ep cells exhibited stratification as well as features of programmed cell death. To determine replicative lifespan, the BC-1-Ep cells were serially cultivated to senescence in standard keratinocyte growth medium at a density of 3.times.10.sup.5 cells per 100-mm dish and passaged at weekly intervals (approximately a 1:25 split). By passage 15, most keratinocytes in the population appeared senescent as judged by the presence of numerous abortive colonies that exhibited large, flat cells. However, at passage 16, keratinocytes exhibiting a small cell size were

evident. By passage 17, only the small-sized keratinocytes were present in the culture and no large, senescent keratinocytes were evident. The resulting population of small keratinocytes that survived this putative crisis period appeared morphologically uniform and produced colonies of keratinocytes exhibiting typical keratinocyte characteristics including cell-cell adhesion and apparent squame production. The keratinocytes that survived senescence were serially cultivated at a density of 3.times.10.sup.5 cells per 100-mm dish. Typically the cultures reached a cell density of approximately 8.times.10.sup.6 cells within 7 days. This stable rate of cell growth was maintained through at least 59 passages, demonstrating that the cells had achieved immortality. The keratinocytes that emerged from the original senescencing population were originally designated BC-1-Ep/Spontaneous Line and are now termed NIKS. The NIKS.TM. cell line has been extensively screened for the presence of specific viral pathogens, including HIV-1, HIV-2, HTLV-1, HTLV-2, HBV, HCV, EBV, CMV, HPV and B19 human parvovirus. None of these viruses were detected. In addition, examination of mice and embryonated eggs inoculated with NIKS.TM. cell extracts demonstrates that NIKS.TM. keratinocytes are free of unidentified viral adventitious agents. The NIKS.TM. cell line is also free of mycoplasma contamination as determined by Hoechst and broth culture.

Detail Description Paragraph - DETX (96):

[0112] In some embodiments, the second messenger assays measure fluorescent signals from reporter molecules that respond to intracellular changes (e.g., Ca.sup.2+ concentration, membrane potential, pH, IP3, cAMP, arachidonic acid release) due to stimulation of membrane receptors and ion channels (e.g., ligand gated ion channels; see Denyer et al., Drug Discov. Today 3:323-32 [1998]; and Gonzales et al., Drug. Discov. Today 4:431-39 [1999]). Examples of reporter molecules include, but are not limited to, FRET (florescence resonance energy transfer) systems (e.g., Cuo-lipids and oxonols, EDAN/DABCYL), calcium sensitive indicators (e.g., Fluo-3, FURA 2, INDO 1, and FLUO3/AM, BAPTA AM), chloride-sensitive indicators (e.g., SPQ, SPA), potassium-sensitive indicators (e.g., PBFI), sodium-sensitive indicators (e.g., SBFI), and pH sensitive indicators (e.g., BCECF).